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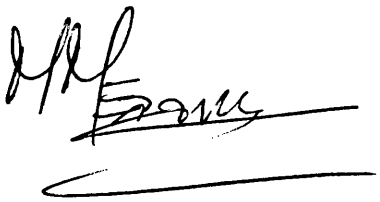
SULPHURED DRIED FIGS IN GREECE: TECHNOLOGICAL ASPECTS AND AFLATOXIN CONTAMINATION

Submitted by Maria A. Melanitou for the degree of Ph.D of the University of Bath 1995



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Abbreviation list

Blue Green Yellow (fluorescence): BGY

Trifluoroacetic acid: TFA

Water activity: a_w

Contents

SUMMARY

The present study was concerned with sulphured dried figs which are a widespread product in the Greek and European market.

One of the achievements of my work was to relate the production side with the ongoing research, by systematizing and controlling the sulphuring, the processing and the packaging of Greek figs in order to obtain a product of good quality with a shelf life of 9 to 12 months. It should be noted that sulphuring procedure and the packaging material have been replaced after our suggestions to the Agricultural Cooperation of Fig Producers - all a result of my experiments.

The effect of sulphuring method and other processing treatments on the sulphur dioxide retained by fig fruits was exemplified by the results of this study. A new simple low cost method for sulphuring Greek figs var. Kymi was developed which resulted in a product with an attractive colour without containing an excess of sulphur dioxide. The factors influencing the loss of sulphur dioxide - whether free, combined or total - throughout storage of dried figs were investigated in a part of this study. It was found that temperature and packaging material exert the greater effect on sulphur dioxide retention.

Moreover, those factors also affect the changes of some physicochemical and microbiological characteristics of packaged dried figs which are important on product stability during storage. The results of this study revealed that sulphured dried figs can be safely stored up to twelve months at 25°C in plastic bags under air providing that initial water activity does not exceed 0.75. A visible deterioration, mainly due to Maillard reaction, occurred only after the ninth month

of storage, indicating thus the inhibitory effect of sulphur dioxide on non enzymic browning.

A survey was also done on fresh and dried figs in order to characterize some microbiological attributes of the product during ripening and throughout storage. It was found that figs when harvested from the tree and properly handled, had a low microbial spoilage. In subsequent storage at 25°C after three months dried figs (either sulphured or not) are practically sterile.

Little attention has been given to the occurrence of toxigenic aspergilli and aflatoxins in dried figs in Greece. In this study it was found that despite the fact that as a substrate fig fruits when ripe supported growth of mold and aflatoxin production, fruits that are harvested from the tree and then sulphured were very unlikely to be infected with toxigenic molds.

These studies also revealed the inhibiting effect of sulphuring on the infection of figs by *Aspergillus flavus* and the production of aflatoxins which is an important problem persisting in non sulphured figs. The effect of sulphur dioxide to degrade aflatoxins B_1 and G_1 was exemplified by the results of this study. However, modern analytical procedures (HPLC) revealed that sulphured dried figs may be contaminated by aflatoxin G_2 but not in levels that could be hazardous to human health.

Future work still needs to be done to elucidate the mechanism of natural infection of figs by *Aspergillus flavus* so that they can be effectively protected. Also future studies ought to be concerned with the role of the sulphur-binding substances of figs to reduce the aflatoxin-degrading effect and the antimicrobial action of bisulphite.

INTRODUCTION

The fig cultivation although not of first importance, is still a source of income for many agricultural families in Greece. The sulphuring of figs for drying although an old practice, is very traditional for some greek fig varieties like that of Kymi. As yet the techniques used for sulphuring were empirical and old fashioned. One of the objectives of this study was the improvement of existing methods so that the quality of the final product can be controlled.

Although there is a long history of production of sulphited dried figs in Greece, there is a paltry number of citations of studies dealing with the technological and other aspects of this commodity. Much work has been done in USA, Turkey and elsewhere. This thesis presents information on three aspects of sulphited Greek figs:

1. The role of sulphur dioxide as preservative agent in dried figs and its behaviour during storage.
2. The shelf-life of dried figs under various conditions of storage as indicated by the rates of physicochemical and microbiological changes of the product throughout storage.
3. The occurrence of aflatoxins in dried figs and the influence of sulphur dioxide to their formation and accumulation.

As far as can be ascertained, the present study is the first of its kind to be done in Greece and provides a foundation of future studies in this country of an important agricultural crop.

CHAPTER 1
LITERATURE REVIEW

The fig tree and its products

History.

The fig is one of the oldest fruits known to man. References to the fig tree were made in the earliest chapters of the Holy Bible. According to Greek mythology, during the War of Titans, Zeus was pursuing Ge and her son Sykeus when Ge, in order to save Sykeus metamorphosed him into a fig tree. An Athenian myth credits the goddess Demeter with having revealed "the fruit of autumn" (the fig), to human beings. One of the earliest references to the fig is made by the poet Archilochus, about 700 B.C, who speaks of fig culture on the Greek island of Paros (Eisen 1901). In the Iliad, Homer refers to the "place of the wild fig tree". Aristophanes, a Greek poet of the 5th century B.C., frequently mentioned the fig in the Acharnians. The word "sycophant" is derived from the Greek sykon, "fig", and phainein, "to show".

The generic name *Ficus* refers to *Ficus ruminalis*, a sacred fig tree which, according to Roman legend, sheltered the infants Romulus and Remus. The specific epithet *carica* refers to Caria, an ancient political division of Asia Minor bordering on the Aegean Sea. Several figs have religious associations including the common fig (*Ficus carica*), which presumably provided raiment for Adam and Eve (Everett 1981). In parts of England, it was common practice to serve fig pies on a certain Sunday in Lent; hence the day was known as "Fig Sunday" (Thiselton-Dyer 1876). In Greece the wild fig was called "erineos" and the edible fig was known as "sykon" from which is derived "syconium", the botanical name of the fruit (Eisen 1901).

Fig tree cultivation.

Botanical evidence points to the fertile region of southern Arabia, as the centre of origin of *Ficus carica*. In Egypt the fig tree was grown before 4000 B.C.. No date has been determined for the time of its domestication but it must have been millennia ago. In the course of time, it spread in cultivation through all the countries of Southwestern Asia and the Mediterranean basin (Storey 1976). The original home of the cultivated fig (*Ficus carica*), conforms closely to that of olive. From its original home it was carried east first to India and then during the Middle Ages to China. It was carried to all parts of the world during the age of exploration and colonization which followed Colombus's discovery of the New World (1492) and circumnavigation of the world by Magelan's ships (1519-21). In that period the garden fig (var. *hortensis*) was introduced into subtropical America and during the half of the nineteenth century the Smyrna fig (var. *smyrniaca*) and the wild fig or caprifig (var. *sylvestris*), together with the insect that effects cross fertilisation, were carried into California (Winton & Winton 1935). Today figs are grown commercially in most of the countries bordering the mediterranean sea, the major producers being Italy, Portugal, Spain, Turkey and Greece. Some figs are grown in California and the southern, drier areas of the USA. In the southern hemisphere, Argentina and Australia have limited production (Jackson 1986).

Agricultural and ecological aspects related to the fig tree.

The common fig belongs to the mulberry family, Moraceae, as the mulberries, the jack fruit and the bread fruit. The fig family is one of the largest in the vegetable world. *Ficus*, is a genus of about 2.000 species of tropical and sub-tropical trees, shrubs and vines (Condit 1969). The fig tree is a large shrub or

low-growing deciduous tree to 10 m tall with large leaves, deeply 3-5 lobed, conspicuously palmately veined, 10-20 cm long (Pursuglove 1968). The fruit requires several dry, warm months for good fruit development. The ideal would appear to be a semi-arid climate with irrigation available. The soil should be well drained and lime free, but otherwise the fig is not very demanding of soil conditions. The life span of a fig tree is short when compared to that of an olive tree. Individual fig trees may continue to thrive up to 100 or more years of age, but most orchard trees decline after 50 to 75 years. Fig trees have a habit of growth or a system of branching, which is more or less characteristic of the variety. The fig tree may carry two crops in a year, depending also on the variety. The first crop is produced from flowers initiated in the preceding late summer. The second crop is produced from flowers on the current season's growth (Jackson 1986).

In recent years, Turkey and Greece have become the largest producers of figs, producing 50000 and 20000 T respectively, during 1975-77, with the USA producing 11500 T and Italy 8200 T during this same period (USDA 1978). The world-wide production of fresh and dried figs goes up to 1200000 T and 200000 T respectively. In Greece, the fig tree has spread to many areas of the country. Especially, fig trees are cultivated in Kalamata, Lakonia and the island of Evia (where Kymi is situated), with the main purpose of producing dried figs. Table 1.1 shows the production of figs per geographical area (Pontikis 1987). The fig industry is largely centred in the provinces of Messenia and Lakonia although some dried figs are produced on the Cyclades, Mitylene, Lesbos, Evia and other islands. The fig orchards near Kalamata, the principal packing and export city, are largely located on terraced hill sides. The trees are planted separately and only occasionally interplanted with grapes or currants. Fertilisation is rarely practised. The annual rainfall varies from 863 to 1219 mm and no irrigation water is added to the soil. Showers in August and September

Table 1.1. PRODUCTION OF FIGS IN GREECE

Geographical area	Production of fresh figs (tonnes)	Production of dried figs (tonnes)
Evia (Kymi)	6910	1033
Kalamata	9299	16627
Ionion Islands	3131	6
Macedonia	2538	562
Aegeon Islands	2259	2528
Crete	2780	212
Totals	26917	20968

Based on Greek Agricultural Statistic Annual Review (1981)

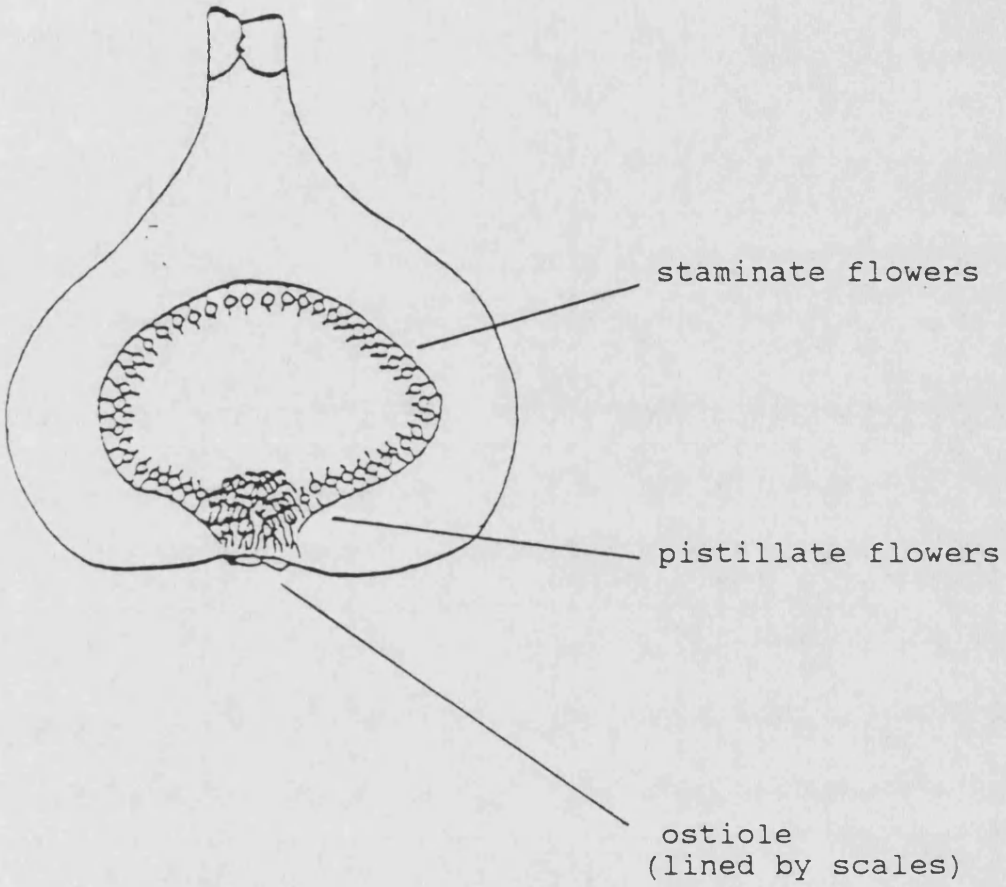
are not infrequent and sometimes cause material damage. Since 1929, there has been in existence a government organisation called the "Office for protection of Greek figs", which is concerned with certain problems of the fig industry. It should be noted though, that there are very few publications concerning fig culture in Greece.

Fig fruits.

The fruiting behaviour of the fig is peculiarly complicated. The fig resembles a multiple fruit but differs in that the individual fruits are not adherent. The fig is unique among fruits in having an apical orifice, or ostiole, which connects the cavity of the receptacle with the exterior (see Fig. 1.1). The so-called "fruit" is the syconium, a complex, enlarged, fleshy, hollow penucle bearing numerous, minute pedicelled flowers on the inner wall. The true fruits are drupelets that develop from the ovaries of the enclosed flowers (Storey 1976). Various parts of the fig tree have a more or less distinctive fragrance. Caprifigs, however, emit a characteristic fragrance which apparently attracts the female Blastophaga to the figs when the flowers are receptive to oviposition (Condit 1932). The fruit at maturity shows a great diversity of colours in the different cultivated varieties - white, yellow, green, red, brown, blue, purple and black. It is more or less pear-shaped with a large soft stem. Around the small opening are small scales. The so-called "seeds" (drupelets) are nearly globular, about 2mm in diameter and hard like berry seeds because of the stony endocarp. These "seeds" vary in size and quantity depending upon the variety. The Calimyrna fig contains a considerable number of large seeds which impart a nut-like flavour to the ripened fruit (Winton & Winton 1935).

The flowers of *Ficus carica* are unisexual. There are two sex forms: the caprifig, which is monoecious and the common fig which is pistillate and

Fig. 1.1. Diagrammatic section of a syconium



requires pollination for fruit development. This is brought about by suspending inflorescences of the wild caprifig in which the fig-wasps, *Blastophaga psenes L.*, are about to emerge on the branches of the flowering fig, a process known as caprification (Pursuglove 1968).

The fruits of fig may be eaten fresh or stewed and they may be dried or canned. Figs are very perishable. They are difficult to transport and cannot be stored even under refrigeration. They are, however, very suitable for processing into canned, dried or frozen products, depending on the variety. There are a multiplicity of fig varieties grown throughout the world. The Smyrna (syn. Calimyrna, Sari Lop, Lob Injir) is a large white fig, principally grown in California and the Mediterranean countries for drying. It has excellent flavour and is attractive when packed. In Greece it is the most common of the varieties but there is also a great number of special varieties, cultivated in some regions of the country. One special variety resembling the Calimyrna which was used in our experiments, is that of Kymi at the island of Evia. It is a medium to large fig, spherical to pear-shaped, yellowish green in colour, has a very thin skin (ideal for sulphuring), red to dark-red inside, excellent quality and requires caprification (See Fig. 1.2a and 1.2b).

Chemical composition

Figs differ in their chemical composition according to varieties, factors affecting the growth of plant such as the growing season, maturity and ripening of figs. Analysis of dried figs harvested at full maturity show less variations than these of fresh figs. A mature fresh fig consists of about 84% pulp and 16% skin. Pellicano (1907) reports that fertile seeds in two common dried figs average 1.02 per cent and those in three Smyrna type figs, 6.49 per cent of the whole fruit. Table 1.2 shows the chemical composition of Greek Calimyrna figs. Figs are

Fig 1.2. (a) A typical fig - variety of Kymi
(b) Cross section of the fig of Kymi

(a)

(b)

recognised as a high energy food especially when dried. Their sugar content increases gradually during the early stages of fruit development and rapidly during the latest stages. The sugar content varies depending upon the variety, with Calimyrnas containing less reducing sugars than the Adriatic, Black Mission and Kadota (Mohamed & Mrak 1942). The glucose content of all fruits was always greater than the fructose, irrespective of variety, crop or harvesting time. Eheart and Mason (1967) determined that the sugar distribution in dried figs was about 50 % glucose, 35% fructose and 10% sucrose. Salem and Abdul-Nour (1979) analyzed dried Iraqi figs (Wazeri variety) and reported per 100 g dry weight: 31.2 g fructose, 34.3 g glucose, 7.8 g sucrose, 1.6 g arabinose, 4.9 g galactose, 79.8 g total carbohydrates and 83.2 g total solids content. Figs also contain non fermentable sugars, two of which were qualitatively determined by Williams *et al.* (1952) to be melibiose and ketoheptose. A sugary material can often develop on the surface of dried figs especially when they are stored at reduced temperatures. This white crystalline material is composed mainly of glucose and fructose. It also contains traces of citric, malic and aspartic acids, as well as lysine and asparagine (Miller & Chichester 1960). Some of the chemical constituents of fresh and dried figs of Kymi and their changes during storage are furthermore discussed in Chapter 4.

Figs contain 3.02 % (dry weight basis) total acids, calculated as malic. Nelson (1928) found that the predominant acid in ripe figs was citric acid. Other acids present in small amounts are fumaric, succinic, malonic, 2-pyrrolidinecarboxylic, oxalic, quinic and glyceric. Acid hydrolysis of this fruit liberated 21 aminoacids. The most abundant amino acid was aspartic acid followed by glutamic acid. The fruit was poorest in methionine and tryptophan (Hegazi & Salem 1977). Fig latex contains ficin a proteolytic enzyme (Walti 1938).

Figs are not recognized as an abundant source of any particular vitamin.

However, they do contain most of the recognized vitamins as well as minerals (see Table 1.2). Their content varies depending upon whether they are consumed fresh or processed. Drying and further processing can result in up to 40 % loss in thiamin, riboflavin and niacin.

The colour of mature figs varies from the white of the Adriatic, the green of the Kadota and the brown of the Calimyrna, to the black of the Black Mission variety. Some studies have been made on the pigments, as well as the carotenoids of some of the varieties (Puech *et al.* 1976). Numerous anthocyanin pigments have been quantitatively identified in figs (Puech *et al.* 1975) such as cyanidin 3-monoglucoside. Cyanidin 3-rhamnoglucoside is the predominant pigment in the skin of ripe Black Mission figs and it also occurs in drupelets of the Kadota and Calimyrna; however, the latter 2 cultivars do not have this anthocyanin pigment concentrated in their skin.

Jennings (1977) has investigated fig volatiles. In recovering and analyzing fig essence he found that the quantitative amounts obtained varied with the degree of ripeness of the figs, with ethyl acetate being the major component. A total of 10 volatile compounds were identified. There seems to be a larger quantitative variation of volatile components within a given variety than there is between varieties. Some of the increase in volatiles in the overly mature fruit could be from a slight fermentation caused by the presence of wild yeasts. The contribution which each of these different naturally occurring chemicals has on the flavour is not known. Dried figs, are a good nutrient and energy source because of their carbohydrate content and the concentration effect caused by moisture removal. Figs are an especially good source of fibre, with fresh figs containing 1.2 % and dried ones 5.6 % respectively.

Table 1.2. Composition of Greek Calimyrna figs

Constituents	Fresh figs	Dried figs
Water*	77.5	23
Calories	80	274
Proteins+	1.2	4.3
Fatty substances+	0.3	1.3
Carbohydrates+	20.3	69.1
Vitamins		
Vitamin A#	80	80
Thiamine B_1&	0.06	0.10
Rivoflavin B_2&	0.05	0.10
Niacin B&	0.4	0.7
Ascorbic acid &	2	0.0
Minerals		
Calcium&	35	126
Phosphorous&	22	77
Iron&	0.6	3.0
Sodium&	2	34
Potassium&	194	640

*: percent of edible part
+: g/100g fresh weight
#: I.U/100g fresh weight
&: mg/100g fresh weight

Based on Pontikis (1987)

Dried figs.

According to Pantastico (1975) other figs for drying should be allowed to fall when ripe and partially dry. The fruits should be frequently picked from the tree to prevent toughening of the skin, mould growth and insect infestation. In most countries this is a hand operation. In the USA the harvesting process is mechanized. Approximately 90 % of the figs in the world are dried (Bolin & King 1980). The interior of a properly dried fig should have the consistency of a thick fruit jam or butter and the skin should have a kid-glove softness and pliability. Occasional stirring or turning the figs during their exposure to the sun and finishing the drying on stacked trays will help to obtain such a product (Condit 1947). Many varieties of figs are sometimes dipped in water and sulphured before being spread in the drying yard. The dipping cleans the fruit and facilitates the absorption of sulphurous acid. The dipped fruit is sulphured for a period of about 4 hours (depending on the method being used) then spread in the sun for 2 to 4 days and drying finished in the stack. The application of sulphuring in figs is examined in detail in Chapter 3 of this project.

Many factors influence storage life of dried figs. SO_2 seems also to play an important role on the shelf life of the product. Chapter 4 of this study deals extensively with that topic.

In recent years, special attention is drawn to contamination of figs by the mould *Aspergillus flavus* group. Some strains of this mould in favourable conditions can produce during storage of the product some extremely toxic metabolites, the aflatoxins. This topic is discussed in detail in Chapter 5 of the present study.

The final part of the literature review is the role of sulphiting agents and microflora of figs along with the occurrence of *Aspergillus flavus* and aflatoxins.

Sulphiting agents

History of use.

As an element, sulphur was well known by the ancients-witness the divine vengeance that befell Sodom and Gomorrah on whom "fire and brimstone" were rained (Genesis). Sulphur was also used for its purifying properties, and from the Greek era until relatively modern times, sulphur was burned for the disinfection which its fumes produced. As quoted by Hammond and Carr (1976), Homer burned sulphur to disinfect his home in ancient Greece. Although not used directly as a food preservative, sulphur was burned sometimes in vessels or places where food or drink was stored. This provides a direct link with modern usage of sulphite as a preservative, because the fumes from burning sulphur contain gaseous sulphur dioxide which is probably the predominant form in which sulphite is taken up by microbial cells to exert its killing action on them (Gould & Russell 1991). There is evidence that the use of the fumes of burning sulphur in wine making was known to the early Egyptians and Romans (Bioletti 1911). The old practice of sulphuring was strictly empirical and based on secular experiences and customs. Sulphuring of fruits for drying was an established practice in the late nineteenth century, in California. In 1902, Beythien and Bohrisch discovered that practically all the dried fruit imported into Germany from America was heavily sulphured and this observation was followed by publication of numerous papers in which the admissibility of sulphur dioxide as a preservative was actively debated.

The inorganic sulphites appeared as food additives at a much later date. Among nonalcoholic products, the sulphiting agents were first used on dried fruits and vegetables in all likelihood. However, their use in foods spread rapidly as a consequence of the absence of toxic hazards and their widespread functional

effectiveness. In the decade between 1960 and 1970, a 30-70% increase in the amounts of several sulphiting agents used annually in the United States was observed (Subcommittee on Review of the GRAS List 1972) a testament to the growing utilization of these additives. Sulphur dioxide was used for preserving meats in the United States as early as 1813 and somewhat later for fish. So widespread was the use of sulphur dioxide in foods in the nineteenth century that Wiley (1907) warned against its promiscuous use as a preservative (Anon. 1907). The widespread use of sulphur dioxide in the dehydration of vegetables during World War II in Britain and the British Dominions and in the United States, focused attention anew upon the problems involved. In spite of its long history of use and the many factors influencing its applicability our knowledge of the chemistry and technology of sulphurous acid and sulphite pretreatment and preservation of foods is still incomplete.

Uses of sulphites in foods.

Sulphite is an unusually multi-functional food additive. Examples of its applications (Table 1.3) include the prevention of oxidation, the inhibition of other chemical and enzymic reactions, use as a bleaching agent, delaying the onset of colour changes in foods, the stabilization of vitamin C, use as a flour-treatment agent and the inhibition of the growth of yeasts, moulds and bacteria (Roberts & McWeeny 1972; Wedzicha 1984). Most of the applications of sulphite are in fruit and vegetable products and in alcoholic and non alcoholic drinks. A small number of applications are in meat products and fish. The fruit, vegetable and drinks applications are exclusively concerned with conserving the organoleptic quality of products, predominantly by delaying the onset of deterioration during storage. Sulphite acts in these applications mostly by interfering with chemical and enzymic changes, e.g in dried, or otherwise water-activity reduced, fruit and vegetable

Table 1.3. Major functions of sulphite in foods

Role	Perceived benefits
Antioxidant	Prevents organoleptic changes due to oxidation of components of foods during storage.
	Minimizes oxidative colour loss of meat and vegetable tissues.
	Aids retention of ascorbic acid (vitamin C) and carotene during storage.
Enzyme inhibitor	Prevents enzymic browning of vegetable tissues due to activity of polyphenol oxidases.
Maillard reaction inhibitor	Prevents non-enzymic browning by forming stable compounds with reaction intermediates.
Reducing agent	Modifies flour rheology via interaction with protein sulphydryl groups.
Antimicrobial agent	Inhibits growth of yeasts and moulds in low pH and low a_w products.
	Inhibits Enterobacteriaceae and other Gram-negative bacteria in higher pH,high a_w products.

Based on Gould & Russel (1991).

products (Schroeter 1966).

Since the functional roles of sulphite in foods vary so widely, the range of applications consequently varies widely too. The major uses are summarized in Table 1.4 in such a way as to illustrate this, with examples of sulphite usage worldwide. It must be emphasized that the foods to which sulphiting agents may be added, and the permitted concentrations, differ greatly from country to country. Furthermore, the levels required for satisfactory preservation may be very food specific. For example, up to 4000 ppm may be necessary to retain the colour of dried apricots during long ambient storage (Woodroof & Luh 1975), whereas in contrast, 250 ppm may be adequate to prevent grey discoloration of potato granules (Sahasrabudhe *et al.* 1976). Sulphite levels in foods are normally quoted as parts per million (ppm) or mg/kg sulphur dioxide (SO_2). However, it is not usual to make use of sulphur dioxide itself, except in applications such as fumigation or during the drying of some cut fruits, but to use sulphur dioxide-generating salts, principally sodium (or potassium or calcium) metabisulphite, and to a lesser extent, salts of bisulphite or sulphite.

Source and application of sulphites.

It has long been believed that fumes of burning sulphur are particularly efficacious in the sulphuring of cut fruits (Nichols & Christie 1930). This method is, though, unsuitable because of uncertainty in application and difficulty in its regulation. However, the use of sulphur fumes in the dried fruit industry is still common, especially in Greece. Cut fruits are treated before sun drying or dehydration with fumes of burning sulphur in specially constructed sulphur houses. The alkali sulphites and bisulphites are also particularly attractive in sulphiting a wide variety of products. Stability, freedom from heavy metal and arsenic impurities, and cost are the chief factors that determine the selection of salts. In

Table 1.4. Major applications of sulphite and levels of use

Typical food uses *	Typical use concentration (SO_2: mg/Kg)
Fresh vegetables (onion, garlic, horseradish pulp)	50-1000
Frozen vegetables (white vegetables, mushrooms)	50
Dried vegetables	250-2500
Canned vegetables	20-100
Pickles	20-100
Peeled potatoes	10-50
Potato powders and flakes	100-500
Frozen potatoes	100
Fresh fruits	100
Dried fruits	100-2000
Fruit pulps, purees and fillings	50-500
Fruit juices	10-100
Jams and jellies	50-100
Fruit-based sauces and related products	50-100
Sugar confectionery	50
Non-alcoholic beverages	20-200
Alcoholic beverages (usually 10-30 in beers, 100-300 in wines)	10-400
Vinegar	50-200
Sausage meat, burgers and other meat products	450
Dried or salted fish	350-1000
Raw or frozen crustaceans	30-350

* Food uses and allowed concentrations vary greatly in different countries
Based on Gould & Russel (1991)

addition, their possible effect in reducing total acidity and introducing metallic cations has to be considered in some products. The sulphites and bisulphites differ in the ease with which they are absorbed and penetrate into fruit and vegetable tissues. Sulphite solutions are useful and better absorbed by apples and other cut fruits that are treated by dipping or immersion before drying (Joslyn & Mrak 1933; Mrak *et al.* 1942b). Commercial sulphiting procedures are extensively discussed by Beavens & Bourne (1945), Woodroof & Cecil (1945) and Cruess (1948).

An alternative source is also liquid sulphur dioxide. Bioletti & Cruess (1912) even before it became available commercially, cited the accuracy with which its doses can be measured and the absence of impurities and prefer it to solutions of sulphur dioxide in water, which they found to be quite variable in strength, corrosive and bulky and inconvenient to handle.

The theoretical yield of sulphur dioxide varies for the different forms of the sulphiting agents. However, it must be realized that these theoretical yields would almost never be achieved in food applications because the sulphiting agents react rapidly with food components, can be volatilized into the atmosphere, or can oxidize to sulphate.

Reactivity of sulphites in foods.

Compared with some of the other widely-used preservatives, for example the organic acids, sulphite is relatively unstable in foods and therefore may lose effectiveness substantially during storage. This is firstly because it may oxidize to sulphate, which is ineffective as an antioxidant, as an antimicrobial, or as a substitute for any of the other roles of sulphite listed in Table 1.3. Secondly, sulphite undergoes many reactions with the molecular components of plant tissues to form products which again do not retain the functionality of the free sulphite. Important in many manufactured foods is the poor stability of many food dyes to

sulphite (Society of Dyers and Colourists 1971). Likewise, anthocyans in natural plant foods may be partially bleached by added sulphite (Banks & Board 1982).

Altogether the chemistry of sulphite reactions in foods has been very extensively studied (Wedzicha 1984) and is discussed below. With regard to preservation, however, its reactivity is important in that initial levels that are present in a food or raw material may often be less easily retained during processing and storage than in the case of other preservatives. Such losses must be taken account of, within legal constraints, in the levels designed in at the stage of product formulation and added during manufacture.

Antimicrobial Activity of sulphur dioxide.

Although not a common practice in the United States, sulphites have been widely used to prevent mould damage in fruits prior to jam or juice production (Roberts & McWeeny 1972; Morris *et al.* 1979). The sulphites are however, selective antimicrobial agents with more inhibitory effect on acetic acid bacteria, lactic acid bacteria and various moulds than on yeasts (Joslyn & Braverman 1954). Roberts and McWeeny (1972) in their review, stated that sulphur dioxide is more effective against the growth of gram-negative rods such as *Echerichia coli* and *Pseudomonas* than inhibiting gram-positive rods such as *Lactobacillus*. Rhem and Whittmann (1962) determined the inactivation levels of sulphurous acid for a variety of yeast genera (Table 1.5). *Torulopsis* and *Saccharomyces* were the most tolerant, while *Kloeckera, Pichia, Rhodotorula* and several other genera were very susceptible to the sulphur dioxide. Dott and Truper (1978) found that "killer yeasts" (those yeasts which when grown in mixed cultures cause the death of other yeasts) were high or medium producers of sulphite and were more resistant to sulphur dioxide. Microorganisms actively take up sulphate ions from the environment, reduce them to sulphite and then to sulphide, which can then be

Table 1.5. Antimicrobial activity of sulphurous acid against various genera of yeasts

Genus	Number of species	Effective concentrations of H_2SO_3 (mg/l) [a]
Saccharomyces	13	0.10-20.20
Zygosaccharomyces	2	7.2-8.7
Pichia	1	0.20
Hansenula	1	0.60
Torulopsis	1	0.20
Candida	2	0.40-0.60

[a] Concentration at which growth and any significant metabolism of substrates ceases.

Based on Rhem & Wittmann (1962)

used in the synthesis of sulphur amino acids. It seems likely that the sulphite moiety produced by reduction of sulphate is not released into the cytoplasm but exists in a complex with a disulphide group of an enzyme (Hammond & Carr 1976). Some yeast strains can also form large amounts of sulphur dioxide during juice fermentations. Very little of the sulphur dioxide formed by the yeast remains in the free state. Sulphur dioxide has also use as a fungicide against a number of moulds that can infect fruits during storage and transit to market, including *Botrytis, Cladosporium, Alternaria, Penicillium, Aspergillus, Rhizopus* etc (Nelson 1979). Several factors are known to control the antimicrobial efficacy of the sulphites. One of the more important factors is pH which controls the form of sulphite present in the food. Apparently, H_2SO_3 is the active form of the sulphites in terms of their antimicrobial action (Ingram 1948; Carr *et al.* 1976), so lower pHs enhance the antimicrobial effect. The combination of sulphites with food components also affects their antimicrobial activity. The sulphite adducts have no antimicrobial activity. Consequently, more sulphite is required to preserve a glucose syrup than a sucrose syrup, since sulphites will combine with glucose but not sucrose (Ingram 1949). The volatilization of sulphur dioxide from acidic products also affects the level retained for antimicrobial action. While sulphonates have decreased antimicrobial activity, several have been found to inhibit yeast respiration (Rhem 1964). The antiseptic action of sulphur dioxide particularly towards yeasts, varies also with the stage of growth or development. The microbial population, temperature and composition of product treated (acetaldehyde content, sugar content, alcohol content etc) is also important. Many other workers (Lochhead & Farrell 1936; Ingram 1948; Hamman 1951; Souci 1951 and von Schelhorn 1953) have stressed the preservative value of free sulphur dioxide.

Sulphur dioxide as an Inhibitor of Enzymic and Non-enzymic Browning.

Sulphur dioxide and sulphites can act as inhibitors of numerous enzymatic reactions, including polyphenoloxidase, ascorbate oxidase, lipoxygenase, peroxidase and thiamine-dependent enzymes. Inhibition of enzymatic browning is the primary reason for using sulphites in many foods (Komanowski *et al.* 1970; Ponting *et al.* 1971; Nelson 1983). As pointed out by Joslyn and Ponting (1951), sulphurous acid and sulphites inhibit the enzyme-catalyzed oxidative discoloration of fruits as a result of mechanical or physiological injury during the preparation for canning, drying or freezing. Under certain conditions when the structure of the fruit tissue and the residual oxygen content of the interior cells permits, it is sufficient to protect the exposed surfaces of the cut fruit against oxidation, by dipping them into, or spraying them with, dilute sulphurous acid or sulphites. In other cases it is necessary for the sulphur dioxide to penetrate the fruit tissue completely, and therefore the concentration of sulphite solution and duration of treatment are important (Ponting 1944). Joslyn and Ponting (1951) suggested that polyphenol oxidases were the chief if not the only enzymes involved in browning of plant tissues. Polyphenoloxidase catalyses the oxidation of mono- and ortho-diphenols to quinones. The quinones can cyclize, undergo further oxidation, and condense to form brown pigments. The role of sulphur dioxide in inhibiting this browning, however, is not known. Sulphur dioxide conceivably could act by reducing oxygen and making it unavailable for oxidation or by reacting with the quinones or other intermediates in polyphenol oxidation. All naturally occurring melanins are conjugated to proteins and Mason (1953) favours interaction of enzymically produced quinones with proteins rather than oxidation of tyrosine residues within the polypeptide chains. If interaction with proteins is also involved in enzymatic browning, then sulphur dioxide could act either upon the enzyme protein or on intermediate products of oxidation. In

products having low a_w, enzyme activity is slow and usually enzymic changes are not the most important ones occurring. It has been found, however, that with dried products, discolouration does occur (Cruess 1948); whether this is sometimes enzymic or is always non-enzymic is debatable, but the presence of sulphur dioxide does retard the browning of some fruits and vegetables in which the enzymes have not been inactivated by any heating process. The ability of sulphites to inhibit the non enzymic browning reactions is largely due to their reactions with the carbonyl intermediates produced in these reactions (Haisman 1974; McWeeny *et al.* 1974). A large portion of the total sulphite residues obtained from such reactions could not be recovered efficiently by the acidic distillation procedure of Monier-Williams (1927). The non-enzymic browning of fruit and vegetable products apparently involves the interaction of sugars, organic acids, amino acids and proteins (Stadtman 1948). There is a strong possibility that the inhibition by sulphur dioxide of browning reactions involving interaction of sugars with amino acids and proteins may be due to the stabilization of the intermediate formed. Hodge (1953) proposed a mechanism for browning in sugar-amine systems based on the Amadori rearrangement in the Maillard reaction (i.e the isomerization of n-substituted aldosylamines to 1-amino-1-deoxy-2-ketones) and stressed the importance of dehydrogenated reductones in both enzymatic and non-enzymatic browning reactions. The known inhibitors for browning include cyanide, dimedon, hydroxylamine, hydrazines, mercaptans, and bisulphite. However, mercaptans and bisulphites, which are the best of the above inhibitors from the practical standpoint, are also reducing agents. Hodge (1953) also suggested that their functions as inhibitors may be related to this property, i.e. their ability to keep the reductones involved in browning in the inactive reduced form rather than the active dehydro form. Reaction of sulphites with the carbonyls generated by non-enzymic browning accounts for most of the loss of sulphites in dehydrated vegetables (Wedzicha *et al.* 1984).

Critical Factors in Determination of the Fate of Sulphites in Foods.

The first critical step in determination of the fate of sulphites in foods is the absorption of the sulphites from dip solutions or sulphur dioxide from the atmosphere into the product. The second factor in determining the fate of sulphites in foods is the nature of the processing treatments. As can be seen from the studies of Thewlis & Wade (1974), Gilbert & McWeeny (1976), McWeeny *et al.* (1980) and Wedzicha *et al.* (1984), sulphite levels can be altered in a number of ways: (1) the sulphites can be physically lost as sulphur dioxide if the pH of the product drops below pH 4.0, especially if the product is heated (2) many of the sulphites in non-acid products can be converted into combined sulphite adducts, many of which remain to be characterized (3) some of this combined sulphite will be in the form of extremely stable products, which cannot be recovered by conventional methods, so it will be "lost" as far as analysis is concerned and (4) oxidation of sulphite to sulphate can occur in some foods and may be particularly significant in wines and flour doughs, perhaps because it is catalysed enzymatically in these foods. The third step in determining the fate of sulphites in foods is the effect of storage on residual sulphite levels. Storage almost always diminishes the amount of inorganic sulphite or free sulphur dioxide in the product. Apparently, processing, storage and preparation act largely to lower the levels of residual sulphite in foods. The actual amounts of free and total sulphite available at the point of consumption have received little study, but it is probable that the lowest free sulphite concentrations would exist at that point.

Sulphites in dried fruits - mode of action.

It is well known that sulphites react readily with reducing sugars, carbonyls and proteins of fruit to yield a variety of organic combined sulphites. The extent of

reaction is dependent on the pH, temperature, concentration of sulphite, and the reactive components of the food matrix. An equilibrium always exists between the combined and free forms of the sulphites, although some of the reactions are virtually irreversible, while others are more readily reversible (Wedzicha *et al.* 1984). These reactions remove free sulphites from the food which often diminishes their effectiveness in the food product. The dissociable, combined forms of sulphite can serve as a reservoir for free sulphite, but the irreversible reactions remove sulphite permanently from the pool of free sulphur dioxide. Burroughs & Sparks (1973 a,b) noted that most of the desirable actions of the sulphites are dependent on the free forms; the combined sulphites are considered to have no retarding effect on product deterioration. Therefore, treatment levels for specific foods have historically been chosen to provide an active, residual level of free sulphur dioxide throughout the typical shelf life of the product. The proportion of added sulphite existing in the combined form would be variable from food to food.
Sulphur dioxide absorption is higher at pH 2.5 than at pH 4.5. Stadtman *et al.* (1946a,b,c) in their studies of the loss of sulphur dioxide measured total sulphur dioxide but Nury *et al.* (1960), when showing that rate of loss was higher from samples with higher initial levels, proposed that this may have been due to the presence of larger amounts of "free" sulphur dioxide.

As noted earlier, the actual levels of free and total sulphur dioxide in the fruit are dictated by the extent of absorption of the sulphites during treatment, the nature of the processing treatment following sulphite addition and the conditions of storage. In a study of the fate of sulphites in dried fruit, McBean (1967) showed that much of the sulphur dioxide was lost on drying and that of the remaining sulphite, 80-90 % was in the combined form. Further losses of sulphite occurred during storage.

Microbiology of figs - Aflatoxins

Microbiology of figs.

Figs frequently undergo an internal yeast fermentation while maturing on the tree. This is possible because botanically the fig is a syconium. It has an opening at the flattened end which is closed by overlapping scales during the early stages of development. After the fruit begins to ripen these scales loosen and an opening termed the "eye" is formed. At full maturity the flesh of each floret becomes juicy, forming an ideal medium for the growth of microorganisms carried into the fruit by various agencies, particularly by insects entering through the "eye" (Mrak *et al.* 1942a).

The major part of infestation takes place while the fig is very young and the eye scales pliable. A high percentage of smut and moulds in figs with sealed eyes probably can be attributed to closing of the eye, which might be expected to create a more humid condition in the fig, favouring germination of mould spores. Most authors discussing fig spoilage (Caldis 1927; Smith & Hansen 1931; Hansen & Davey 1932) refer to yeasts in connection with a type of deterioration termed "souring"; a spoilage involving the production of acid and a vinegar-like odour. Smith & Hansen (1927) described souring as a form of spoilage causing the contents of ripe figs to ferment and sour with subsequent dripping of liquid from the "eyes". Condit (1933) stated that souring is caused by the action of specific yeasts and bacteria on the internal sucrose juice of the fig. Investigation has shown that figs are internally sterile until they are entered by insects, after which they commonly become infected with yeasts, moulds and bacteria. Caldis (1930) indicated that fig souring is primarily an alcoholic fermentation but subsequent changes may take place; the commonest being that brought about by the action of acetic acid bacteria on the

alcohol with the production of acetic acid. He isolated three types of wild yeasts, producing typical souring which were termed as *Mycoderma, Apiculata* and *Torulaspora*. Davey & Smith (1933) included *Mycoderma, Pseudosaccharomyces, Hanseniaspora* and *Pichia* species in the true souring yeasts of figs. The non-souring yeasts included those forming a membranous, wrinkled, dry, surface growth on solid media. Mrak *et al.* (1942a) isolated 115 cultures of yeast from soured figs which represented species of *Saccharomyces*, *Pichia* and the apiculate yeasts (*Hanseniaspora* and *Kloeckera), Candida* and *Torulaspora*.

Two striking changes may occur in the microflora of Calimyrna figs due to involvement of insects. The first change is a normal one and it occurs when the green sterile fruit is pollinated by *Blastophaga* wasps (Phaff & Miller 1961). The constant association of a pale tan and a pink bacterium with the fig wasp, with the caprifig and with pollinated Calimyrna figs was first recognised by Caldis (1927). He also concluded that the colourless bacterium corresponded to *Achromobacter nitrificans* Bergey and the chromogenic strain appeared to be related to *Serratia marcescens* Bizio, although it differed in a number of ways. Smith and Hansen (1931) reported that they obtained yeast colonies when the pulp of freshly dried, commercially sound passable Calimyrna figs were plated. These included figs of perfect appearance, odour and flavour. Phaff & Miller (1961) found that the microflora of the caprifig consisted of a single bacterial species, *Serratia plymuthicum* Bergey, and a single species of yeast, *Candida guillermondii* var. *carpophila.* It was shown that the fig wasp introduces these microorganisms into the Calimyrna variety during pollination. Despite a limited multiplication in the interior of the fig, these organisms do not cause a detectable fermentative spoilage or souring. Miller & Phaff (1962) estimated the population distribution of the significant spoilage organisms. The most common organisms were apiculate yeasts (*Hanseniaspora*

valbyensis and *Kloeckera apiculata), Candida krusei* and *Candida guillermondii* var. *carpophila*. The apiculate yeasts and *Torulopsis stellata* comprised at least 90 % of the spoilage flora of figs. This finding was in contrast to those of Mrak *et al.* (1942a) and Miller & Mrak (1953), where *Saccharomyces cerevisiae* and *Candida krusei* were listed as the most numerous species present in spoiled figs. When caprifigs are improperly handled by the grower, they may become infected with mould. As a result the fig wasp may also carry spoilage moulds to the edible figs. One of the most troublesome moulds carried by the fig wasp is *Fusarium moniliforme* var. *fici* (Caldis 1927). This fungus causes an internal rot of the syconium known as endosepsis.

The second change in microflora follows the visitation of the fruit by other insects during the later stages of ripening or in dried fruit. These are *Drosophila melanogaster* and *Carpophilus hemipterous*. Invariably, after the fruit was visited by *Drosophila*, fermentative spoilage and souring ensued. During ovipositing in the fig, the fruit is contaminated with yeasts and bacteria adhering to exterior body parts of the flies. During subsequent larval development, the larvae spread the contaminants throughout the flesh of the fig and active fermentative spoilage results. There is a difference in opinion as to whether the dried fruit beetle feeds on fig fruit tissue or the fungi (particularly yeasts) occurring in the fruit, or both. Phillips *et al.* (1925) and Caldis (1927) indicated the beetles feed on fruit tissue whereas Smith & Hansen (1931), expressed the view that the beetles are mycophagous and enter the fig in search of fungus foods. Regardless of the reason for the insects entering the fig, the result of their entrance is direct or indirect damage to the fruit. The beetle normally lives in the soil or in decomposing plant material. Ordinarily, it does not seem to carry sufficient spoilage yeasts and bacteria to initiate souring, unless it originated in a fig already undergoing fermentation (Miller & Mrak 1953). The latter situation may occur during the final stages of ripening. Different bacteria and especially a

short rod in chains were usually associated with the yeasts or obtained as the soil flora. *Aspergillus* sp., *Rhizopus sp.* and *Penicillium sp.* were also obtained from beetles, the first by far the greatest number of times. Phillips *et al.* (1925) in their studies of the transmitting agents of *Aspergillus niger* causing what is called black smut in figs consider *Carpophilus hemipterous* first in importance and *Drosophila ampelophila* as second.

Microorganisms occurring during drying and storage of figs.

In many districts (ed. California) it was customary to harvest figs for drying after they have already undergone a partial drying in the orchard. They were usually gathered from the ground at frequent intervals and carted to fumigation champers as soon after picking as possible, for the purpose of killing insects and insect eggs present. Unfortunately, this treatment, as used in commercial practice did not free the fruit of living yeasts, moulds and bacteria (Natarayan *et al.* 1948). Microbial spoilage frequently occurs in figs while maturing on the trees and sometimes while drying on trays on the field. Spoilage during drying is more apt to occur in the cooler districts or during periods when the weather is unfavourable. There is little information available relative to the microbial changes occurring during drying. Nichols & Reed (1932) concluded that dehydration reduced the spoilage of figs by insect infestation and microorganisms, but a marked reduction of such spoilage was possible only when figs were picked from the tree. When dried in the sun, on the other hand, the green colour disappears and the fruit attains an attractive amber colour.

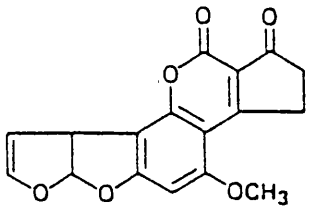
Dried figs commonly become covered with a white sugary substance during storage before processing and occasionally in cartons after processing. It is generally believed that this substance is composed of sugar crystals. Microscopic examinations of "sugared" dried fruit samples have shown that this

substance consists of a mixture of yeast cells and sugar crystals (Baker & Mrak 1938). The genera and sub-genera of the sporulating yeasts isolated were in the order of their frequency of occurrence: *Zygosaccharomyces, Hansenula, Saccharomyces, Debaryomyces* and *Zygopichia*. Several cultures of imperfect yeasts and a few cultures of bacteria were also isolated. No obligate osmophilic yeasts were encountered.

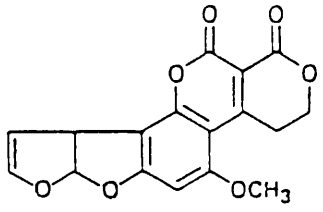
Aflatoxins.

The fact that fungi or moulds produce unpleasant flavours or other undesirable changes in foods has been known for a long time. In addition, some moulds also have the capacity to form chemical substances that are poisonous or produce toxic symptoms when food containing them is ingested by man or animals. These chemicals are known as mycotoxins and the diseases caused by consumption of these fungal-contaminated foods or feeds are known as mycotoxicoses (Buchi & Rae 1969). Interest in mycotoxins was stimulated by the discovery that the death of 100000 turkeys at 500 locations in England in 1959 was due to a toxic, carcinogenic fungal metabolite produced by the fungus *Aspergillus flavus* being present in the protein supplement in the feed (Lancaster *et al.* 1961). The name aflatoxin had been given to the toxic metabolites of *Aspergillus flavus* before it was appreciated that it was a complex mixture of compounds and before their structures had been elucidated (Moreau & Moss 1979). In fact, most strains of *Aspergillus flavus* group which are toxigenic produce mainly aflatoxin B_1 with smaller amounts of aflatoxin G_1, B_2 and G_2. Chemically, aflatoxins are difuranocoumarin derivatives with B_2 and G_2 being the dihydro derivatives of B_1 and G_1 respectively (Fig. 1.3). The term mycotoxin stems from two Greek words: "mykes" meaning fungus and "toxicum" meaning poison (Goldblatt 1972). Examples of mycotoxins produced by moulds are shown in Table 1.6. The letters B and G refer to the fluorescent

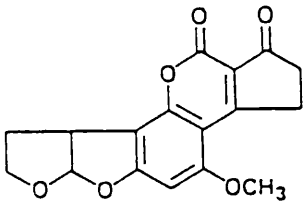
Fig 1.3. Chemical structure of the aflatoxins



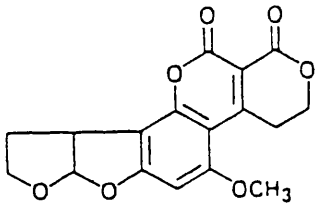
Aflatoxin B_1



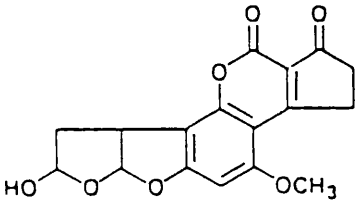
Aflatoxin G_1



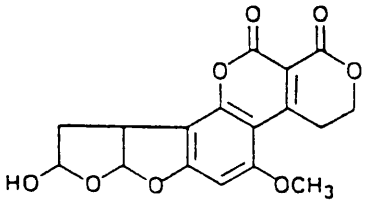
Aflatoxin B_2



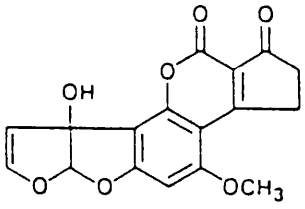
Aflatoxin G_2



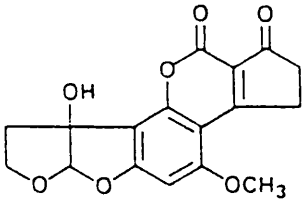
Aflatoxin B_{2a}



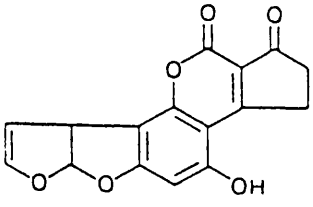
Aflatoxin G_{2a}



Aflatoxin M_1



Aflatoxin M_2



Aflatoxin P_1

Table 1.6. Examples of Mycotoxins produced by Molds

Molds	Mycotoxins	Minimal a_w * for growth	for toxin produc.
Aspergillus flavus	Sterigmatocystin Aflatoxins Aspergillic acid Kojic acid Aspertoxin	0.78-0.80	0.83
A.fumigatus	Gliotoxin		
A.niger	Oxalic acid		
A.ochraceus	Ochratoxins Penicillic acid	0.77 0.76	0.85 0.81
A.versicolor	Sterigmatocystin		
Penicillium citrinum	Citrinin		
P.expansum	Patulin		
P.patulum	Patulin	0.81	0.83
P.viridicatum	Citrinin		

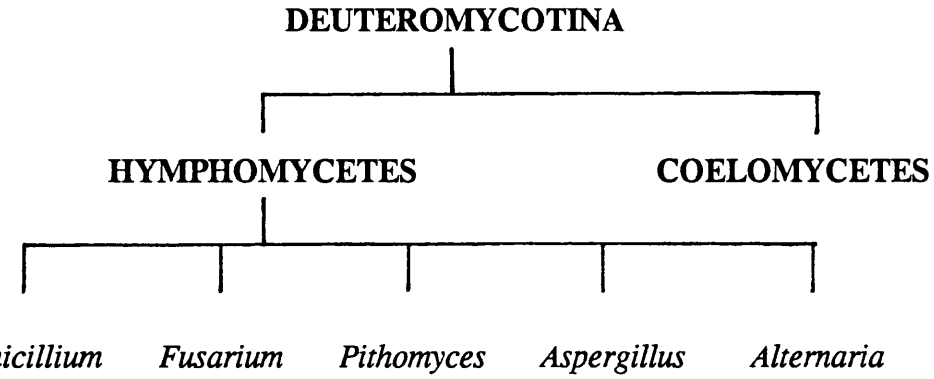
Based on Yabuta 1924, White 1940, Menzel *et al.* 1944, Bullock *et al.* 1962, Kuehn & Gunderson 1963, Holzapfel *et al.* 1966, Rodricks *et al.* 1968, Van Walbeek *et al.* 1969, Diener & Davies 1970, Hutchinson & Holzapfel 1971, Scott *et al.* 1972, Harwig *et al.* 1973, Sommer *et al.* 1974, Reiss 1975, and Troller 1980.

* a_w: water activity

colours observed under long-wave ultra-violet light and the subscripts 1 and 2, to the separation patterns of these compounds on thin layer chromatographic plates (Bullerman 1979). The genus *Aspergillus* belongs to a class of fungi known as the *Hyphomycetes*, which belongs to the subdivision of fungi known as the *Deuteromycotina*. The *Hyphomycetes* consist of organisms with conidia produced directly on mycelia or on single or clustered conidiophores (Fig. 1.4, Beuchat 1987). The *Aspergillus flavus* group of species is a normal constituent of the microflora in air and soil and is found on living or dead plants and animals throughout the world (Raper & Fennell 1965, Fig.1.5). Most moulds, which are capable of producing aflatoxins, are also frequent contaminants of food and agricultural commodities. Apart from *Aspergillus flavus* and *Aspergillus parasiticus, Aspergillus nomius*, a new aflatoxigenic species phenotypically similar to *Aspergillus flavus*, has been recently isolated (Kurtzman *et al.* 1987). Shih & Marth (1973) studied the location of aflatoxin and noted that a significant amount of aflatoxin was retained by the mycelium of *Aspergillus flavus* and thus was not in the substrate when the mould culture was harvested.

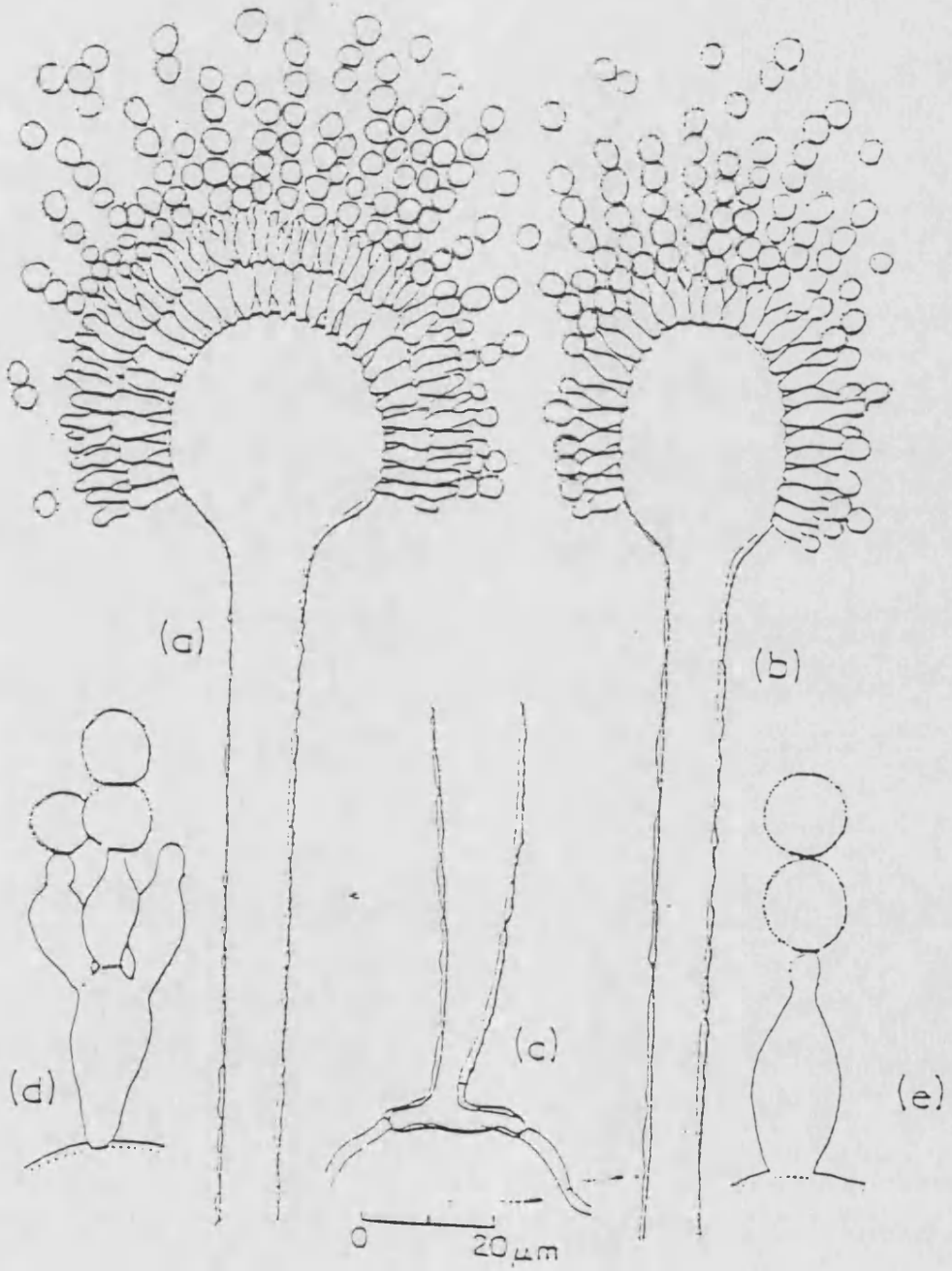
Aspergillus species are capable of growing on a variety of substrates and under a variety of environmental conditions. The presence of aflatoxigenic moulds on a substrate does not automatically mean the presence of aflatoxin. Conversely, the absence of aflatoxigenic moulds does not guarantee the absence of aflatoxins, since the toxins may persist long after the mould growth has disappeared. The natural contamination of commodities is likely to persist whenever warm and moist weather conditions, faulty or inadequate storage facilities, and human error combine, to produce circumstances favourable for fungal growth and toxin production (Diener & Davis 1969). At the 1st FAO/WHO/UNEP Conference on mycotoxins in 1977, a review was presented of the occurrence of mycotoxins in various commodities throughout the world (Food Agr. Organ. Un. Nat. 1979).

Fig. 1.4. Classification of molds.



Adapted from Beuchat 1987

Fig. 1.5. *Aspergillus flavus* Link
(a) sporing head with biseriate phialides
(b) sporing head with uniseriate phialides
(c) base of conidiophore
(d) detail of biseriate phialides
(e) detail of isolated phialide.



Only 7 mycotoxins were found to occur significantly in naturally contaminated foods and feeds: aflatoxins, ochratoxin A, patulin, zearalenone, trichothecenes, citrinin and penicillic acid. The information on aflatoxin contamination exceeded that for all other mycotoxins; likewise, most concern was expressed about aflatoxins. Furthermore, regulations for the control of aflatoxins in food exist in at least fifty different countries throughout the world and maximum amounts are specified from zero tolerance (in practice, the detection limit of the method) up to 50 μg/kg (Van Egmond 1989).

Occurrence of aflatoxins in foods.

Aflatoxin production on substrates has been reviewed by several workers (Hesseltine *et al.* 1966; Wildman *et al.* 1967; Diener & Davis 1969). *Aspergillus flavus* produced aflatoxin experimentally on over 25 fruit and vegetable juices and other foods. Aflatoxins have been found in many agricultural products (Sanchis *et al.* 1986) such as stored peanuts (Golumbic 1965; Sherertz *et al.* 1976), corn (Shotwell *et al.* 1969), oats, sorghum and wheat (Ramakrishna *et al.* 1990), cottonseed (Ashworth *et al.* 1968), hazelnuts and haricot beans (Lötter & Kröhm 1988). Also, aflatoxins have been produced on cured wet tobacco leaves and crushed black sunflower seeds inoculated with *Aspergillus flavus* (Pattee 1969; Llewellyn & Eadie 1974).

Aflatoxin production on any given substrate will vary quantitatively with the strain of the fungus, temperature, moisture in the substrate and/or surrounding relative humidity, aeration, length of the incubation period and the method of aflatoxin analysis. The most important factors influencing growth and aflatoxin production are relative humidity surrounding the substrate, which in most cases is between 88 and 95 % and a storage temperature of 25 °C to 30 °C (Bullerman

1979). Studies have been concerned with the invasion by *Aspergillus flavus* of living fruits destined for drying and it was not completely clear whether the fungus is a pathogen of fruit as well as of seed. Whether *Aspergillus flavus* can parasitize living fruit tissue is a critical question. If *Aspergillus flavus* is a fruit pathogen, infection, colonization and aflatoxin production would be expected in the orchard. If it is a strict saprophyte, one must look for faulty or inadequate methods of handling, drying, or storage, which permits fungus colonization of the dried commodity. Other carbohydrate-rich fruit, such as pineapple and cooked apricot, were recognized as good substrates for aflatoxin production by Morton *et al.* (1979). In recent years, contamination of food products in the home has been studied. Of interest is the possibility that mouldy foods may produce aflatoxins. Unprocessed fruits and vegetables support *Aspergillus* moulds under both refrigerated and non-refrigerated conditions. There has been no reported occurrence of aflatoxins in apricots, pineapples, or raisins. However, with the indications that some dried foodstuffs and figs in particular, have been contaminated with aflatoxins (Anonymous 1974), studies were undertaken to evaluate their potential for contamination by aflatoxins under experimental conditions (Buchanan *et al.* 1975, Altug 1988).

Factors affecting aflatoxin biosynthesis.

Aflatoxins have been shown to be produced by *Aspergillus flavus* and *Aspergillus parasiticus* species. However, the types of toxin produced are species specific. *Aspergillus flavus* produces mainly aflatoxins B_1 and B_2, while *Aspergillus parasiticus* produces aflatoxins B_1, B_2, G_1 and G_2. Furthermore, not every species or strain produces aflatoxin (Hesseltine *et al.* 1985) as the genotype of each strain determines whether or not it is aflatoxigenic. Competing mould microflora affect aflatoxin production through competition for substrate and through the production

of inhibitory metabolites. Competing mould microflora may either metabolize the aflatoxin produced, alter the metabolism of *Aspergillus flavus*, compete for substrates necessary for aflatoxin formation, or render conditions unfavourable for aflatoxin production (Moss & Frank 1985b; Moss & Smith 1985). The type of substrate also affects aflatoxin synthesis and levels of aflatoxin production. Substrates high in proteins and low in carbohydrates do not enhance aflatoxin production by *Aspergillus parasiticus*. However, *Aspergillus flavus* can utilize low amounts of carbohydrates and produce substantial amounts of aflatoxin (Park & Bullerman 1983). With prolonged incubation at temperatures of approximately 25 °C, proteins are degraded to amino acids by fungal proteases. Some of these amino acids serve as nitrogen sources, while others serve as a source of carbon, if other carbon sources are limited. When amino acids are used as carbon sources, large amounts of ammonia may be liberated, which affects aflatoxin production, as ammonia and other readily metabolized nitrogen sources depress or inhibit aflatoxin production (Diew & Demain 1977). Studies have shown that optimum aflatoxin production occurs on solid substrates rich in carbohydrate such as coconut, wheat, rice and cottonseed (Arseculeratue *et al.* 1969; Detroy *et al.* 1971). Aflatoxin biosynthesis and level of production is also influenced by the nutrient composition of the substrate. Simple sugars such as glucose, fructose and sucrose are the preferred carbon sources for aflatoxin biosynthesis by *Aspergillus flavus* (Davies & Diener 1968). Mannose and xylose have been shown to stimulate aflatoxin production by *Aspergillus parasiticus*. However, these two sugars have been shown to inhibit aflatoxin production by *Aspergillus flavus* (Mateles & Adye 1965). Certain compounds, which inhibit fungal growth, also affect aflatoxin biosynthesis. Propionic acid is an effective antifungal agent against *Aspergillus flavus*, but its activity is influenced by pH and certain feed ingredients (Dixon & Hamilton 1981). Sorbic acid is also a food preservative, and its salts have been shown to inhibit growth and aflatoxin production by *Aspergillus flavus* and *Aspergillus parasiticus*

(Yousef & Marth 1981).

The optimum temperature range for fungal growth and aflatoxin production was found to be 25 °C to 30 °C (Northolt *et al.* 1977). As a result of temperature variation, the yield of aflatoxins can vary considerably. At low temperatures, approximately equal amounts of aflatoxins B and G are formed, while at higher temperatures aflatoxin B production predominates relative to aflatoxin G. Several studies have shown that aflatoxin can be produced at temperatures as low as 7.5 to 10 °C (Schroeder & Hein 1967; Van Walbeek *et al.* 1969). Low temperature storage can be very suitable for controlling the growth of *Aspergillus flavus*. However, temperature should be reduced to 5 °C as quickly as possible throughout the product, especially in perishable commodities. Even though refrigeration temperatures may be an ideal means of controlling mould growth, care should be taken not to turn it into an ideal environment for aflatoxin production. Minimal growth of *A. flavus* will occur by maintaining temperatures at 0 °C. The optimum a_w for aflatoxin production by both *Aspergillus flavus* and *Aspergillus parasiticus* is reported to be in the range of a_w 0.95 to 0.99 (Diener & Davis 1969). At low a_w, water is bound by salts, sugars, proteins and other solutes, therefore growth of moulds cannot occur since water is not present in an available form (Northolt *et al.* 1976). Aflatoxin production ceases or decreases at a_w values below 0.85. However, fungal growth can still occur at a_w values as low as 0.78 to 0.80. The minimum a_w for growth of *Aspergillus flavus* has been reported as 0.78 to 0.84, while the minimum a_w for toxin production was found to be 0.84 (Table 1.7).

The atmosphere of storage also affects growth of *Aspergillus* and aflatoxin production because fungi are aerobic organisms. Low concentrations of carbon dioxide have been shown to be beneficial to spore germination and are involved in fungal metabolism and in the synthesis of proteins, nucleic acids, and intermediates of the tricarboxylic acid cycle (TCA). However, concentrations of CO_2 greater than

Table 1.7. Effect of a_w * on spoilage of foods by microorganisms

a_w	Probable spoilage microorganisms	Food
0.90-1.00	Bacteria	Cottage cheese, Fresh meat
0.85-0.90	Bacteria and molds	Margarine
	Yeasts	Sweetened condensed milk
	Bacteria	Whipped butter
0.80-0.85	Yeasts	Chocolate syrup, fruit syrups
0.75-0.80	Xerophilic molds	Dried figs
	Molds and yeasts	Jams
0.70-0.75	Yeasts	Confections
0.65-0.70	Osmophilic yeasts	Molasses
0.60-0.65	Xerophilic molds	Dried fruit
	Osmophilic yeasts	Honey

Adapted from Troller 1980

* a_w: water activity

20% inhibit mould spore germination, while more than 10% CO_2 suppresses toxin production (Tabak & Cooke 1968). A decrease in atmospheric oxygen (O_2) to less than 20%, or increase in oxygen concentration to 90% or higher, have also been shown to inhibit aflatoxin formation (Landers *et al.* 1967; Shih & Marth 1973). While carbon dioxide has been shown to increase the lenght of the lag phase and hence decrease the rate of mould growth (Baker *et al.* 1986) there is a little or no data with respect to aflatoxin production in food packaged under CO_2-enriched atmospheres. Bennett *et al.* (1978) showed that aflatoxin production was inhibited by light at either high or low temperatures but not at intermediate temperatures between 20 to 25° C. Joffe & Lisker (1969) observed that aflatoxin production was completely inhibited in Czapek's medium in the presence of light, while Reiss (1975) showed that light had no effect on aflatoxin production in bread.

Lie and Marth (1968) reported that *Aspergillus flavus* and *Aspergillus parasiticus* were able to grow over a range of pH values from 1.7 to 9.34, with optimum growth occurring between pH 3.42 and 5.47. However, aflatoxin production did not occur at all pH levels.

Due to the increasing number of reports on the toxic nature of this chemical, there was a need to prevent contamination of products by aflatoxin-producing moulds or to control mould growth by manipulation of their microenvironment. Other control methods were directed at either reducing the concentration of aflatoxins to safe levels or to producing nontoxic degradation products without reducing the nutritional value of the treated commodities (Doyle *et al.* 1982). Treatment of aflatoxin-contaminated cottonseed meal with ammonia to detoxify this product is approved in some states in the U.S. and in other countries. A common means of avoiding aflatoxin in agricultural products is to dry the commodity without delay to a water content that will not support growth of the causal fungi. Another promising experimental process is treatment of aflatoxin -contaminated substrates with bisulphite (Doyle & Marth 1978a, b). This topic will be discussed

in Chapter 5. Moerck *et al.* (1979) observed that ammonia, sodium hydroxide and bisulphite destroyed aflatoxin in corn after 24 hours at ambient temperature and they reported that bisulfite was effective at 0.5 and 1% levels. Hagler *et al.* (1982) confirmed these results, but degradation of aflatoxin in corn by bisulfite was slower than noted earlier; thus, more severe conditions and a longer time were required to achieve degradation comparable to that reported by Moerck *et al.* (1979). Sreenivasamurthy *et al.* (1967) found that hydrogen peroxide (H_2O_2) together with alkali destroyed 97% of aflatoxin B_1 in peanut meal. Altug *et al.* (1990) used ultraviolet (UV) radiation to degrade aflatoxin in milk. They found that destruction of the toxin by UV radiation was greater in the presence rather than absence of H_2O_2.

I would like here to stress the fact that my experiments were done under very difficult circumstances. A part of my work had to be done in Kymi by using the very poor equipment of the Agricultural Cooperation. Most of the methods of analysis used in this work, were developed by myself as no one at the Institute had worked with those till now. This is the reason why some of them were chosen relying on their simplicity and low cost. Importantly, some of the methods were designed for application by the Greek farmers because the initial purpose of this Institute is to connect research with production and so the relationship between researcher, research and farmer is extremely important.

CHAPTER 2
Materials and Methods

A. MATERIALS

All chemicals used are of the maximum possible analytical purity. Solvents used for High Performance Liquid Chromatography were of HPLC grade. Some special chemicals used are as following:

bromophenol blue (indicator pH 3.6-4.6): 0.4 g of bromophenol blue (Merck) are dissolved in 6 ml of 0.1 M sodium hydroxide (tritrisol Merck) and the mixture is diluted with water to 100 ml.

Silica gel for column chromatography (for 50 gram samples of figs): Silica gel 60 (Merck), 0.063-0.2 mm, is activated by drying for 1 hour at 105° C, then water is added (1 ml/100g), sealed, shaken until thoroughly mixed and stored for more than 15 hours in an air-tight container.

Phosphate buffered Saline (PBS): Potassium chloride (0.2 g), potassium dihydrogen phosphate (0.2 g), anhydrous disodium hydrogen phosphate (1.16 g) and sodium chloride (8.0 g) were added to approximately 900 ml of distilled water and were gently agitated until all had dissolved; after adjustment to pH 7.4 (with 0.1 M sodium hydroxide or hydrochloric acid) the solution was made up to 1000 ml with distilled water.

Primary Aflatoxin standards (Sigma chemicals): mixture of the four aflatoxins $B_1+B_2+G_1+G_2$ in benzene:acetonitrile (98:2 by volume) at concentrations of 5 ppm for B_1 and G_1 and 1.5 ppm for B_2 and G_2. Also individual standards (1 mg of each aflatoxin) were used. The appropriate dilutions were made (0.2-100 ppb) to

find the detection limit of the High Performance Liquid Chromatographic method. Concentration of solutions was determined by UV absorbance (Association of Official Analytical Chemistry 1984c).

Derivatized aflatoxin standards (according to Tarter *et al.* 1984): the appropriate quantity of the individual aflatoxin solutions made from primary standards, was tranfered to a vial, to obtain the following amounts of aflatoxins: B_1, G_1 = 500 ng, B_2, G_2 = 100 ng. After evaporation to dryness under a gentle stream of nitrogen, 200 μl hexane and 50 μl TFA (via Eppendorf pipette) were added, capped and mixed with Vortex mixer for 30 s and were let to stand 5 min. 5.0 ml water-acetonitrile (9:1 by volume) solution were added and mixed for 30 s. Layers were let to separate for 5-10 min. Final concentration is B_1, G_1 = 0.5 μg/5.05 ml and B_2, G_2 = 0.1 μg/5.05 ml (in lower aqueous phase).

Packaging materials and procedure.

It is known from the literature (Stadtman *et al.* 1946a,b,c) that oxygen availability is one of the factors that influences the rate of darkening and sulphur dioxide loss of dried fruits during storage. The test-pack treatments were designed to assess the relative role of major packaging variables on the loss of sulphur dioxide from sulphured fruits packaged in flexible films.

Polyethylene was chosen as one of the package materials because it is known to be highly permeable to both oxygen and sulphur dioxide (Davies *et al.* 1973).

The plastic bags used for the experiments were obtained from *Multipack* corporation (a Greek company): one from low density polyethylene high barrier to water vapour and actual permeability to oxygen ca 35, carbon dioxide ca 90 and nitrogen ca 9 $ml.m^{-2}.day.bar$ at 20°C (plastic A) and the other from a high density

polyethylene-polypropylene laminate high barrier to water vapour and actual permeability to oxygen ca 15, carbon dioxide ca 35 and nitrogen ca 9 $ml.m^{-2}.day.bar$ at 20°C (plastic B). In order to obtain the above permeabilities, the LPDE films were relatively thick (thickness of 430 mm).

The packs were sealed by means of a Henkovac 1700 automatic sealer equipped with a gas supply. The packs were filled with figs (100 g) then evacuated to a pressure of 5 cm Hg, gas flushed and heat - sealed. Two gas-flushing treatments were used: 100 % air and then 50 % carbon dioxide-50% air.

Fig supply.

The figs used for the experiments were obtained from the Agricultural Cooperative from Kymi area - Evia, Greece from September to November during 3 harvesting periods (1989-1991-1992). The figs of Kymi belong to the Calimyrna variety, but are of a special kind with thin tissue which permits a satisfactory penetration of sulphur dioxide. They are marketed in paper packages of 500 g (Figure 2.1).

Fig 2.1. Package used at market for the figs of Kymi.

B. METHODS

Section A: Sulphur dioxide; improving methods of sulphuring greek figs and effect of several factors on loss of sulphur dioxide through storage (Chapter 3)

1. Methods of sulphuring.

The methods used for sulphuring figs were the following:

1) Burning sulphur (powder obtained from the market) in an oven.

2) Spraying the figs with SO_2 solutions of various concentrations (while the fruits were exposed to sunlight).

3) Dipping figs into SO_2 solutions of various concentrations and for various times.

4) Exposing figs to vapour produced from a SO_2 solution.

5) Direct gas sulphuring from a bomb of SO_2.

A more detailed description of the methods is given at the Materials and Methods of **Chapter 3.**

2. Determination of total sulphur dioxide.

Although only a proportion of the preservative (the undissociated fraction of the unbound sulphurous acid) is effective against microorganisms, the maxima stated in the regulations relate to the total amount present (calculated as SO_2 w/w). Maximum permissible limits of SO_2 are stipulated by most countries for dried foods and for fruits these range from 2000 to 3000 parts per million (mg/kg).

A. Monier - Williams distillation method.

The total SO_2 content of the figs was determined by the Shipton's modification of Monier-Williams distillation reference method (Shipton 1954). The sample is distilled with acid and the sulphur dioxide is absorbed with excess of an oxidising agent, which converts it into sulphuric acid. The apparatus used is shown in

Fig.2.2. The main modifications from the earlier method are: (a) the use of a less cumbersome vertical reflux condenser with ground-glass joints; (b) the replacement of gas by electrical heating; (c) the use of a regulated flow of nitrogen as inert gas because carbon dioxide may interfere with the end point; (d) the abolition of the necessity of using previously boiled water; (e) the use of un neutralised hydrogen peroxide, which can be corrected for by means of a blank titration; and (f) the omission of the heating of the condenser at the end of the refluxing.

Preparation of sample.

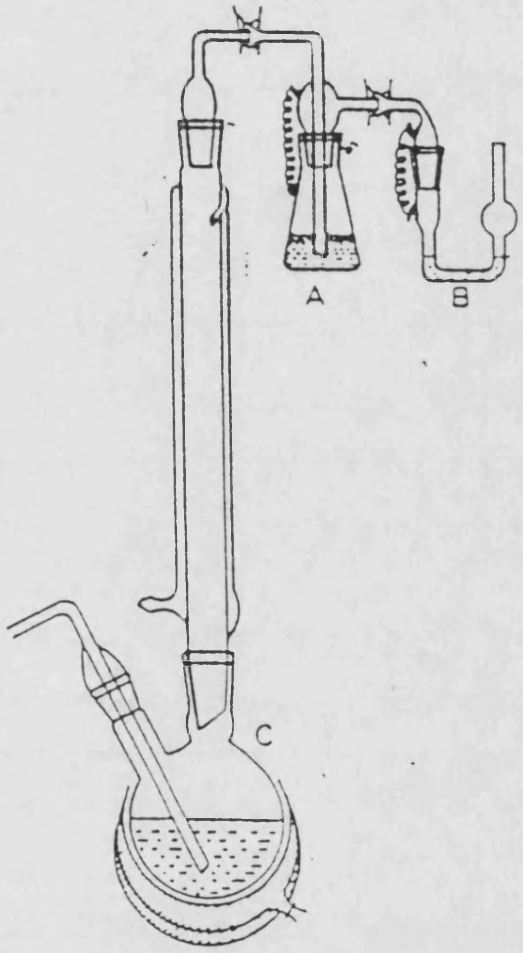
Approximately 20 Kg samples are taken from different trays (one layer of figs in each tray) widely distributed throughout the factory. The size of the sample was chosen in order to eliminate the great variability in the amounts of SO_2 absorbed by each fruit. The entire sample was finely cut by passing twice through a 9 mm meat mincer (Butcher Boy TM 20) at normal speed. The resulting paste was homogenized and sub-samples of 20-25 g are taken for subsequent analysis with an estimated 3-40 mg of SO_2.

Procedure.

After connecting up the apparatus, ensuring that all joints are greased and tight when clamped, the flask C is surrounded with a circular electric heating mantle. The flask A and the U-tube B are removed and in each one was placed 15 ml and 5 ml respectively of 3% hydrogen peroxide (w/v). Water is added to each so that bubbles pass through. The prepared sample is added to C, with 350 ml of water and 20 ml of concentrated hydrochloric acid.

Immediately, the inert gas supply was connected adjusting the rate of flow to

Fig. 2.2. Shipton's apparatus for the determination of sulphur dioxide; the sample is boiled with acid in flask C and the sulphur dioxide produced is absorbed in hydrogen peroxide contained in flask A and guard-tube B.



6-12 bubbles per minute in the U-tube. After switching on the electric heater to full heat and the liquid boils, the heat was turned down to give a slow, steady boil. The boiling is continued for 30 min, ensuring that the rate of flow of gas is maintained. Then the conical flask and the U-tube are disconnected and the solution is transferred in the latter to the former, washing it in with a little water. The combined liquids in the flask are titrated with 0.05 M sodium hydroxide using bromophenol blue as indicator. The free acid was titrated in a further 20 ml of the hydrogen peroxide solution (blank titration). The SO_2 in parts per million (ppm) was calculated following the equation:

$$SO_2 = \frac{\text{(Sample titration-Blank titration) x 1600}}{\text{weight of sample taken (g)}}$$

where 1600 is a factor due to the fact that 1 ml of 0.05 M sodium hydroxide is equivalent to 0.00160 g sulphur dioxide.

Recovery.

The check on recovery of procedure was made as follows:
An approximately 1% sodium sulphite solution was prepared; 10 ml of 0.1 M iodine solution were pipetted into a conical flask and 1 ml of 5 M hydrochloric acid and 40 ml of water were added. 10 ml of the approximately 1% sodium sulphite solution were run in slowly from a pipette, swirling the flask throughout the addition to ensure completion of the reaction, then the excess of iodine was titrated with 0.1 M sodium thiosulphate, adding starch solution as indicator towards the end. The weight of sulphur dioxide (as SO_2) contained in the 10 ml of solution taken was calculated considering that 1 ml of 0.1 M iodine = 0.003203 g SO_2.

Then 10 ml of sodium sulphite solution was added to the distillation apparatus along with 350 ml of water and 20 ml of concentrated hydrochloric acid and the

procedure of the distillation was carried out. From the titration with standard sodium hydroxide, the weight of sulphur dioxide which has reacted was calculated and the result was compared with the weight obtained from the iodine titration. The recovery of the method as described above was 87 %.

B. Colorimetric method.

The colorimetric method of Association of Official Analytical Chemists (1984a) was also used for the determination of total SO_2 content of the dried figs, but only in purpose to make a comparative study between the two methods (colorimetric versus distillation).

3. Determination of free and bound SO_2.

Joslyn and Braverman (1954), in reviewing an extensive literature on the chemistry and technology of SO_2 utilization for treating fruits and vegetables, pointed out that in the treated products this preservative must exist in both free and combined forms, the relative proportions of these being determined by such factors as the composition of the food, its pH and its temperature, all of which will play a part in determining the final state of equilibrium attained.

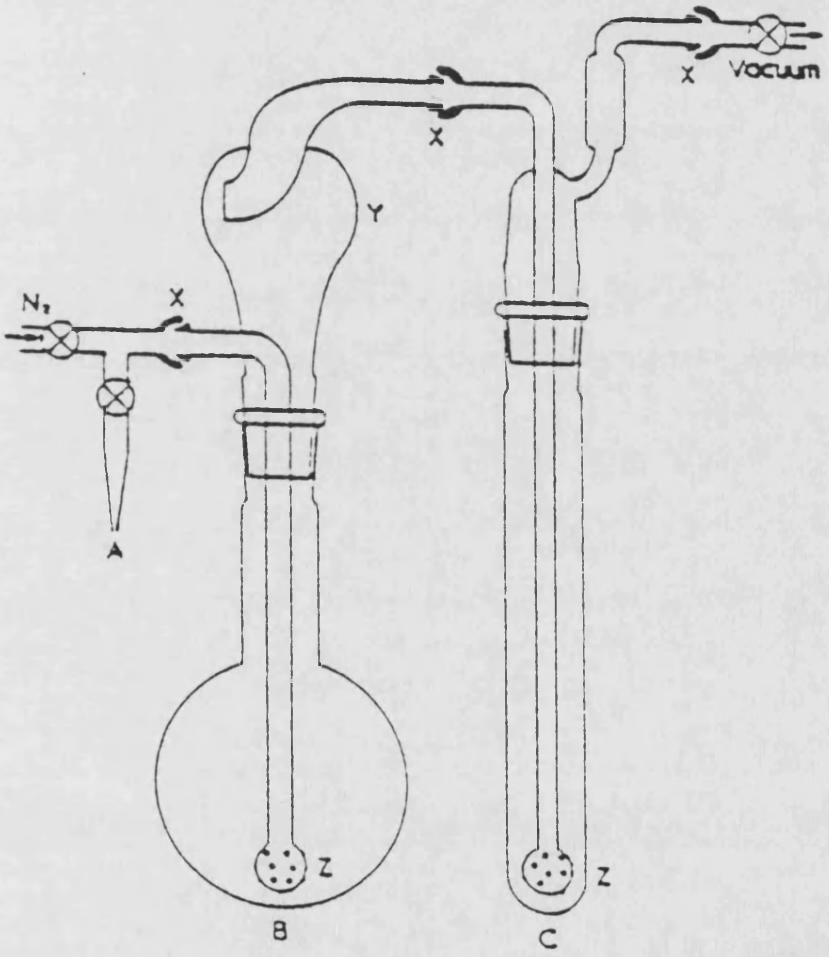
The free or uncombined SO_2 in sulphured dried figs was determined by the method developed by Burroughs and Sparks (1964) as it was modified by McBean (1967). The following minor modifications were made to the method of Burroughs and Sparks:

1) The apparatus used was as shown in Fig. 2.3. A fine-bore pressure relief tube with tap A was installed to prevent sucking back. The splash-head Y prevented carry over of phosphoric acid from the sample flask B to the absorption tube C, and the fine bubblers, Z, assisted in ensuring efficient absorption of SO_2 from the emergent stream of nitrogen. Ball joints at X facilitated assembly and a standard pressure of 50 mm Hg was used throughout.

2) The hydrogen peroxide was used at 3% strength (10-vol), because SO_2 levels found in sulphured fruit are higher than in ciders.

3) Bromophenol blue was used as an indicator, as in the Monier-Williams method.

Fig. 2.3. Equipment for determining free sulphur dioxide in sulphured fruit; sample is put at flask B and displaced free sulphur dioxide is transferred by emergent stream of nitrogen and absorbed by 3% hydrogen peroxide solution at tube C.



4) Caprylic alcohol (0.5 ml) was added to flask B to reduce frothing.

Fruit was blended in 25 % orthophosphoric acid in water to lower its pH to between 1 and 2, thereby largely suppressing dissociation of combined SO_2 during displacement of the free SO_2 for subsequent estimation. The sample is put in flask B and displacement was accomplished by drawing a stream of oxygen-free nitrogen through subsamples of the blended fruit at room temperature by applying suction at 50 mm Hg (vacuum). After 15 minutes tube C is replaced and drawing of nitrogen is continued for another 15 minutes. The SO_2 so displaced was absorbed from the emergent nitrogen stream by 3% hydrogen peroxide solution at tube C and estimated by titrating the resulting sulphuric acid with standard sodium hydroxide solution 0.05 M with bromophenol blue as indicator. Whatever SO_2 was absorbed by tube C during the second 15 minutes, is supposed to come from the dissociation of bound SO_2 and gives the appropriate correction of the free SO_2 displaced from sample during first 15 minutes period. The combined SO_2 is determined at the residue left in flask B by the Monier-Williams method. The amount so determined, was added to the dissociated combined SO_2 which was trapped during the second 15 minutes period. Determinations of free and combined SO_2 were done on individual samples of fruit and at the same time corresponding samples were taken for analysis of total SO_2 content.

According to McBean (1967), a single 15-min period of nitrogen bubbling through the sample, was adequate for the displacement of free SO_2 from the sample under test and also, under the conditions used, no significant displacement of combined SO_2 occurred from sulphured fruit during this operation. In view of probability that in any case the principal SO_2-combining agent in fruits is glucose, and that very little dissociation of SO_2-glucose occurs at pH 1.5 (Vas 1949) blank corrections such as those used by Burroughs and Sparks were not used.

It is impossible to prevent entirely, some loss of SO_2 through volatilization or oxidation during the above analytical procedures, particularly in the determination of free SO_2 where fruit is blended in phosphoric acid (Ponting & Johnson 1945). However, the use of a carefully standardized sampling procedure and short blending times are believed to have restricted such losses to insignificant amounts.

Section B: Studies of physicochemical and microbiological changes that occur during commercial and cold storage of dried figs (Chapter 4)

A. PHYSICOCHEMICAL ANALYSIS

1. Determination of moisture.

The moisture of dried fig samples was determined according to the gravimetric method described by Moysidis (1957). According to that method, 1.4 g of Hyflo super cel (Serva)-Kieselguhr was mixed well with 3.5 g of very well ground figs and a little hot water (for the best mixing of sample with the super cel). The mixture was placed at an aluminium capsule (70 mm diameter, 20 mm height). The capsule was placed over a steam bath and heated until all the added water was removed and the capsule (with the sample) gained its initial weight (before the addition of hot water). That is why the capsule was removed from the steam bath from time to time and weighed. After that, the capsule was left for 75 min more over the steam bath, then wiped with a clean cloth and further dried in a desiccator over calcium chloride and weighed. The moisture content of the sample was calculated from the difference of weight. 10 determinations of each sample were made with an estimated error of the method $\pm$ 0.6 %.

2. Determination of colour.

The colour measurements were made with a Hunterlab colorimeter Model D25-PC2Δ (lamp voltage=9.29) in L, a, b scale. The "L" measures lightness and varies from 100 for perfect white to zero for black; "a" measures redness when plus, gray when zero and greenness when minus; "b" measures yellowness when plus, gray when zero and blueness when minus (Grncarevic & Lewis 1973). In our work the instrument was standardized against a yellow calibrated ceramic tile.

Samples were presented in a dish (10 cm diameter and 2 cm deep) with the sample surface always levelled.

3. Development of browning.

The extend of browning was assessed by measuring the absorbance at 440 nm of ethanolic extracts of the tissue according to Nury *et al.* (1960). A 15-gram sample of well mixed ground dried figs was placed in a 300-ml Erlenmeyer flask containing 200 ml of 50 % ethanol. The flask was then covered with parafilm and allowed to remain at room temperature for 23 hours with occasional shaking. The coloured solution was filtered through Whatman No.2 filter paper and the colour reading made with a Carlo Erba computer controlled spectrophotometer (Spectracomb 602) with a digital plotter, at 440 nm using a 1.2 cm cell. The results were recorded as absorbance (A=log 1\T), with a 50 % ethanol solution used for zero adjustment.

4. Measurement of sugars.

The general volumetric method of Lane-Eynon was used (Association of Official Analytical Chemists 1984b) for the measurement of reducing and invert sugars. The samples (dried figs sulphured and not) were maintained at constant temperature (4°C and 25°C) under a relative humidity of 70 %. One package of each sample was withdrawn at each sampling time. Duplicate measurements of the sugars were made.

5. Determination of water activity.

Water activity was determined with the Thermoconstanter Humidat-TH2 (Novasina, Zurich, Switzerland). The fig samples for analysis were cut into small pieces and filled into the sample bowls provided by the manufacturer. The sample bowls were put into the measuring chamber at the preselected temperature (25°C).

The equilibrium relative humidity was read off the display in % RH when the value remained unchanged for 10 minutes (equilibration time) and the indicated temperature corresponded to the preselected value. Water activity was calculated as : a_w = % equilibrium relative humidity / 100 (Labuza 1982).

B. MICROBIOLOGICAL ANALYSIS

Twenty five grams fresh or dried (sulphured and non sulphured) figs were immersed in 225 ml of distilled water and macerated with a stomacher (Colworth, UK) at high speed for 60 seconds at room temperature. For **AFPA** and **DG18** media the time needed was 40 seconds at normal speed in order to avoid fragmentation of mycelia. The following counts were made, by spreading, in duplicate, 1 ml or 0.1 ml samples of decimal dilutions on the media indicated: (the culture media used for microbiological tests were all obtained from OXOID Ltd, except acetobacter agar which was made from basic ingredients in the laboratory)

a) for general viable count and cultivation of microorganisms.

-PCA (*Plate Count Agar) CM325*, a medium for the enumeration of viable organisms in milk and dairy products, but also used for general viable count and cultivation of microorganisms. Incubation time is 3-5 days at 20±3°C.

b) For the cultivation and enumeration of yeasts and moulds.

-RBC (*Rose-Bengal Chloramphenicol Agar) CM549*[1], for the selective enumeration of moulds and yeasts from a wide variety of foodstuffs. Chloramphenicol used to suppress the growth of bacteria. Incubation time is 5 days at 22±2°C.

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[1]with supplement-chloramphenicol SR78

-DG18 (*Dichloran-glycerol Agar Base) CM729*, a selective low water activity medium (a_w) for xerophilic moulds from dried and semi-dried foods. Incubation at 25°C-examination after 4, 5 and 6 days.

c) For identification and enumeration of acetic acid bacteria:

- **Acetobacter Agar** (Vanderzant & Splittstoesser 1993) made per litre from: autolyzed yeast (10.0 g), calcium carbonate (10.0 g), agar (10.0 g) and glucose (3.0 g). Incubation temperature is 30±2 °C - with examination every day until the seventh day. *Acetobacter* colonies cause clear zones in an otherwise cloudy lawn in $CaCO_3$.

Also the characteristic oxidation of ethanol to CO_2 + H_2O by *Acetobacter* was used as identification test (Marakis 1990). For this purpose, a medium made from yeast extract (30 g), agar (20 g) and a solution of 2.2 % (v/v) of bromocresol green (1 ml) was made in 1 l of distilled water. To the substrate was then added 20 ml ethanol at 15°. Microorganisms were inoculated at the centre of petri dishes and incubated at 28°C for 3-4 days. *Acetobacter* spp. produced acetic acid and changed the colour of the indicator from green to yellow. Further oxidation to CO_2 + H_2O changed colour from yellow to blue-green.

Section C: Aflatoxins in Greek dried figs (Chapter 5)

A. MICROBIOLOGICAL ANALYSIS

The culture media used were obtained from OXOID Ltd, except APA which was made from basic ingredients in the laboratory:

a) for the identification and enumeration of *Aspergillus flavus* group:

-Czapek Dox Agar (Modified) *CM97*, a solid defined medium for the general cultivation of fungi and bacteria, specially of those which are able to utilize sodium nitrate as the sole source of nitrogen. The incubation time (for *Aspergillus*) is 1 week at 30±2°C.

-AFPA *Base CM73*[2] (Pitt *et al.* 1983, Hocking & Pitt 1986), a selective identification medium for the detection of *Aspergillus flavus and parasiticus*, which are potential aflatoxin producers. Incubation for 42-48 hours at 30°C is required.

b) for detection of aflatoxin-producing strains of *Aspergillus flavus*:

-APA (Aflatoxin producing agar) medium (Hara *et al.* 1974); aflatoxin producing strains of *Aspergillus flavus* were detected by fluorescence of a modified **Czapek's** solution agar under ultraviolet light. The composition of the medium used was (per 1 litre): potassium hydrogen phosphate (1 g), magnesium sulphate-7 H_2O (0.5 g), potassium chloride (0.5 g), ferrous (II)sulphate-7 H_2O (0.01 g), sucrose (30 g), agar (20 g), ammonium dihydrogen phosphate (10 g), mercuric chloride ($5x10^{-4}$M) and distilled water (1 L); the pH was adjusted to 5.5 with 1M sodium hydroxide before the addition of agar. Strains were inoculated at the centre of the solidified agar medium in glass petri dishes (fluorescence cannot be detected in plastic dishes) and incubated at 28°C in the dark. These were examined under ultraviolet illumination (360 nm) from the seventh through the tenth day of incubation for the presence or absence of blue fluorescence in the agar surrounding the colonies.

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[2] with supplement chloramphenicol SR78

c) for maintaining *Aspergillus* cultures:

-**PDA** (*Potato Dextrose Agar) CM139* and Czapek Dox Agar.

The strain of *Aspergillus flavus* group used for inoculation, was the toxigenic *Aspergillus parasiticus* Speare ATCC 26862 which is known to produce aflatoxin B_1, B_2, G_1 and G_2 and is isolated from soil (Wei & Jong 1986).

B. PHYSICOCHEMICAL ANALYSIS

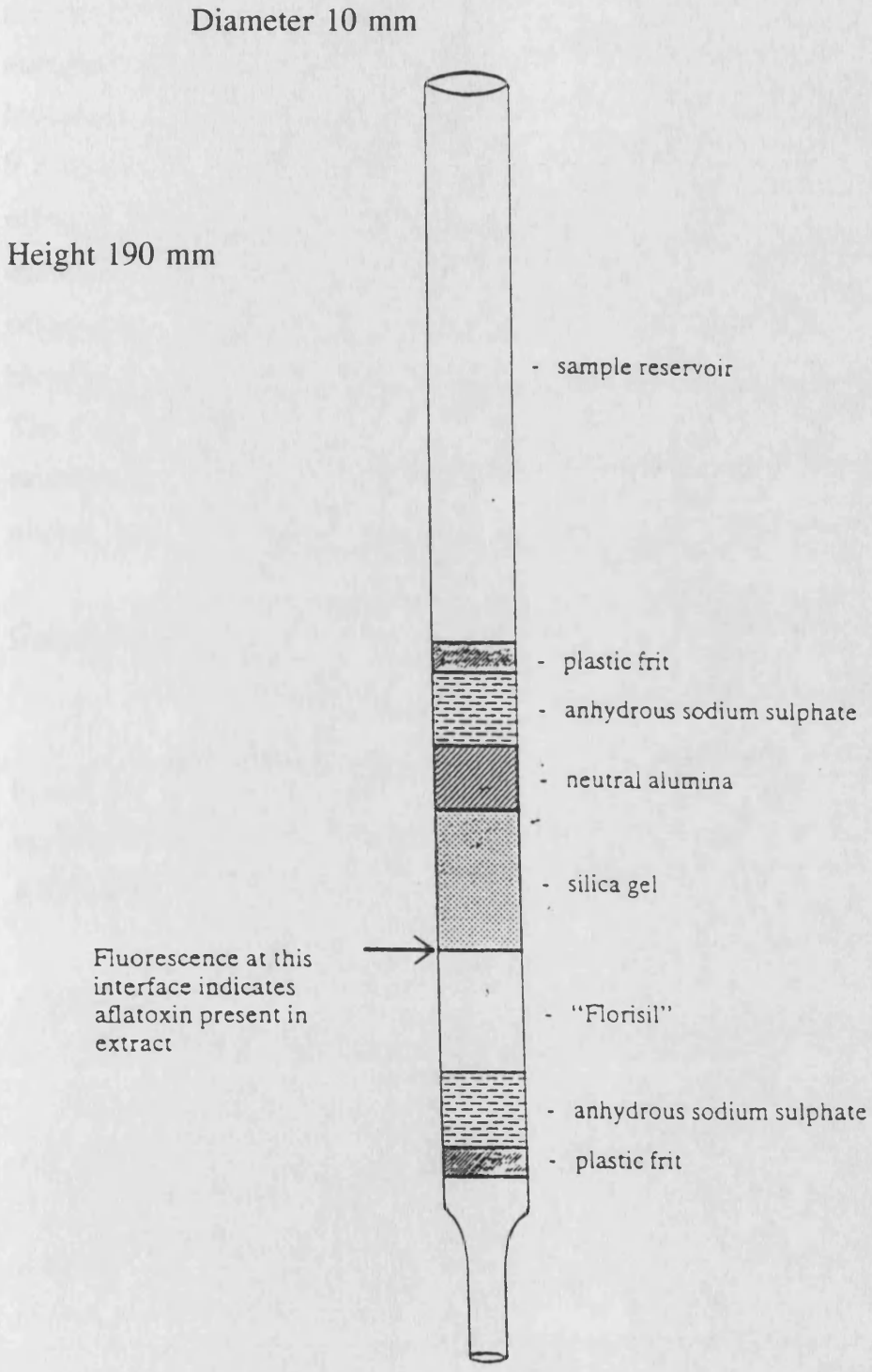
1. Detection of aflatoxins.

The aflatoxins were detected semi-quantitatively by the *Total Aflatoxin Test kit TD 100* of Biocode (York, UK) and quantitatively by a High Performance Liquid Chromatographic method. The extraction of aflatoxins for the HPLC was carried out as described in *AOAC-Official methods of Analysis (1984c)* and in *Working Group on Toxins-Mit. Geb. Lebensm. Hyg. (1982)*

a. Total Aflatoxin Test Kit.

This is a rapid semi-quantitative screening method for the extraction and detection of total aflatoxin. Extraction was carried out using the *EASI-EXTRACT Immunoaffinity column TD101*. The extracted aflatoxin was then passed through the *Detection Minicolumn (TD102)* (Figure 2.4) which contained a band of a particular silica known to augment the natural fluorescent property of aflatoxin. The extracted aflatoxin may then be viewed under long-wave ultraviolet light. The detection limit of the kit was 1 ppb (1 μg/kg).

Fig. 2.4. Construction of detection minicolumn (TD 102) used for the detection of aflatoxins in fig samples.



Sample preparation /Dilution of Extract.

The minimal sample size to be taken from consignments of figs was 20 kg to be made up of a number of sub-samples of equal weight. To gain a representative sample of a batch, a number of 30 g portions were collected from different locations within the batch. The sample particle size was reduced by grinding with a 9 mm meat mincer (Butcher Boy TM 20) at normal speed. The sample is then effectively mixed to obtain homogeneity and sub-divided in lots of 10 g. It was essential to analyze sufficient samples from a batch since aflatoxin contamination often occurs in pockets within the bulk material. 10 g of ground sample was blended at high speed with 20 ml acetonitrile/water (3:2 by volume) for 2 minutes. The blended sample was then centrifuged at approximately 800 g (average) for 10 minutes at room temperature. The extract supernatant of filtrate was retained and eluted appropriately with phosphate buffered saline.

Column Preparation.

The empty barrel of a disposable 20 ml syringe was clamped vertically above a beaker to collect waste. 10 ml of phosphate buffered saline was placed in the syringe barrel. The syringe plunger was used to push this through the *EASI-EXTRACT* column at a slow steady rate such that droplets were formed.

Aflatoxin extraction.

The *EASI-EXTRACT* column was loosened from the syringe body and, was filled with the diluted extract and this was pushed through the column at a slow steady rate with the plunger. The column eluent was discarded, then washed with deionised distilled water. This elute was also discarded. The aflatoxins were finally eluted with 2 ml methanol and the eluent was collected in a glass stoppered vial.

Aflatoxin detection.

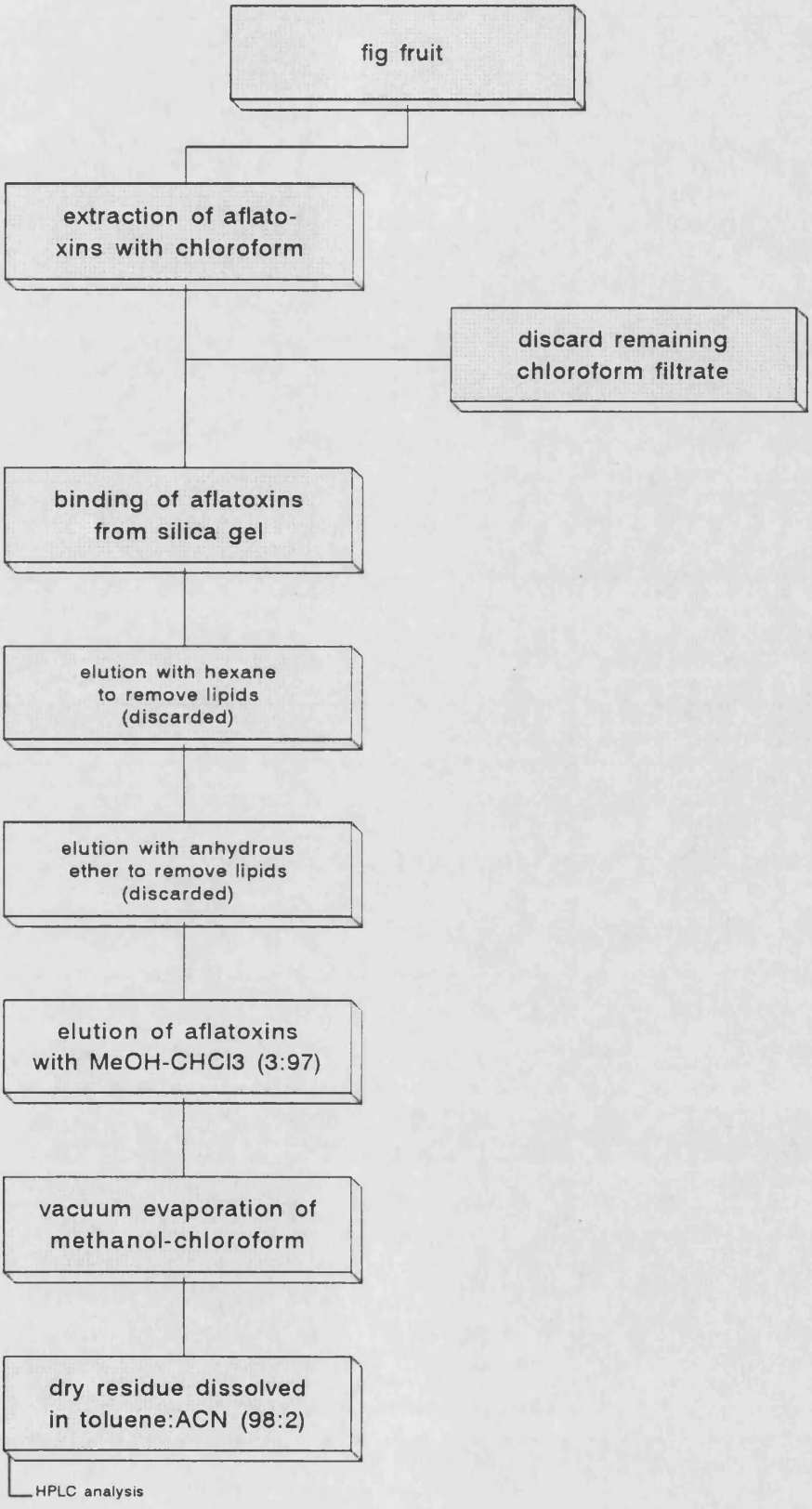
To the column eluent was added 6 ml distilled water and 3 ml chloroform, the vessel was stoppered and shaken vigorously (releasing pressure occasionally). The mixture was left stand, to allow the layers to separate. If any emulsion was formed, this could be resolved by centrifugation at 800 g (average) at room temperature for 5 minutes. The lower organic layer was removed with Pasteur pipette and applied to the *Detection Minicolumn*. Liquid was allowed to run into the column bed material under gravity. 3 ml of a chloroform/acetone (9:1 by volume) solution was added to column and allowed to pass through under gravity. When the meniscus of the solvent reached the top plastic frit, the column was removed from the clamp and viewed under ultraviolet light at 360 nm in the dark, preferably in a dark box. If a fluorescent band was clearly visible at the *'Florisil'* silica gel interface (see Fig 2.4), total aflatoxin ($B_1+B_2+G_1+G_2$) was present in the sample at, or greater than 1 ppb. With the four fluorometric standards supplied by *Aflatest-Rhone Poulenc,* equivalent to 0-5-10-20 μg/kg (ppb) aflatoxin, a subjective quantification can be made if the sample is positive for aflatoxins by comparing the fluorescence of sample with that of the standards. If no fluorescent band was visible at the *'Florisil'*/silica gel interface, then total aflatoxin was either completely absent, or present at levels below the defined level (i.e 1 ppb).

b. High Performance Liquid Chromatographic analysis.

Preparation of sample - extraction of aflatoxins (for 50 g).

The figs were well homogenised with a blender. 50 g of prepared sample were weighed into a 500 ml Erlenmeyer flask. 25 ml water was added, along with 25 g diatomaceous earth and 250 ml chloroform and the stopper was secured with masking tape and shaken. The resulting suspension was filtered through paper. The first 50 ml portion of this chloroform filtrate were collected and the column chromatography performed (Fig. 2.5).

Fig. 2.5.Flow chart for extraction of aflatoxins from fig fruits



Column chromatography.

A 22x300 mm chromatographic tube was used with a filter of glass wool at the bottom and a teflon stop-cock. 5 g of anhydrous sodium sulphate was added at the bottom to give base for the silica gel; next chloroform was added until the tube is half full; then 10 g silica gel 60 (0.063-0.2 mm) was added. The sides of tube were washed with 20 ml chloroform and stirred to disperse the silica gel. When the rate of settling slows, some chloroform was drained to aid settling and about 5-7 cm of chloroform was left above the silica gel. Slowly, 15 g anhydrous sodium sulphate was added and some chloroform was drained to the top of the sodium sulphate layer. 50 ml sample extract was added to this column and allowed to elute with a rate of 1-3 ml/min, then eluted at maximum flow rate with 150 ml hexane followed by 150 ml anhydrous ether and discarded. The aflatoxins were eluted with 150 ml mixture methanol-chloroform (3:97 by volume) by collecting this fraction from time of addition until flow stops. The sample was evaporated nearly to dryness on steam bath (by rotavapor) and quantitatively residue was transferred to vial with chloroform and evaporated preferably under gentle stream of nitrogen. The vial was sealed with hollow polyethylene stopper, capped and saved for HPLC analysis (in freezer). To the vial containing sample extract, 200 μl of toluene-acetonitrile (98:2 by volume) was added and resealed with polyethylene stopper, shaken vigorously to dissolve preferably with a vortex shaking machine.

TFA derivatization.

200 μl hexane and 50 μl TFA was added to extract in vial, capped and mixed with Vortex mixer for 30 s, let stand 5 min and then 950 μl water-acetonitrile (9:1 by volume) was added via Eppendorf pipette and mixed for 30 s. Layers were left to separate 10 min and lower aqueous phase was transferred in a new vial and analysed the same day. Sample concentration was 10 g/ml of aqueous acetonitrile.

Liquid chromatographic determination.

The method used for the HPLC analysis was a modification - combination of the one used for aflatoxins in figs by Food Science Laboratory of Ministry of Agriculture, Fisheries and Food of United Kingdom (personnal communication 1989) and the one described by Tarter *et al.* (1984). It was a reverse phase liquid chromatography with previous TFA derivatization, with the use of a C_{18} column, (protected by a guard column) and fluorescence detection. The instruments and the analytical conditions are described in detail in materials and methods of **Chapter 5.** Using instrument parameters, successively 25 μl of derivatized standard solution and 25 μl of TFA-treated sample solution were injected. Aflatoxin concentration was calculated as follows:

B_1 or G_1, μg/kg = [sample peak ht (area)/standard peak ht (area)] x (0.5 μg/5.05 ml) x (1.0 ml/10 g) x 1000

B_2 or G_2, μg/kg = [sample peak ht (area)/standard peak ht (area)] x (0.1 μg/5.05 ml) x (1.0 ml/10 g) x 1000. The detection limit of the method was 0.1 ppb

Confirmation.

Analysis was repeated on a second 50 g portion of sample, collecting the final fraction from the column and evaporating to dryness in the vial on a steam bath. 200 μl hexane and 950 μl water-acetonitrile (9:1 by volume) was added via an Eppendorf pipette, mixed for 30 s, the layers were left to separate for 10 min and 25 μl of the lower aqueous layer was injected into the LC apparatus. The absence of peaks with same retention times as B_{2a} or G_{2a} confirms the absence of interference which, if present, do not form derivatives with TFA, but would have the same retention times as B_{2a} and G_{2a}.

The statistical analysis of variance was done with the MINITAB (Minitab Inc, USA 1988) and STATGRAPH Ver. 6.0 (Manugistics Inc, USA 1992) software. Also the CURVEFIT Ver. 2.10 (Imtec, USA 1988) software packaging was used. The error bars, where in the figures, represent the standard deviation.

CHAPTER 3

Improving methods of sulphuring Greek figs and effect of several factors on sulphur dioxide loss

Introduction

The chemical reactions of sulphur dioxide when it is added to fruits are complex and many of them are still unknown, though considerable research has been done in this area (Joslyn & Braverman 1954; McWeeny *et al.* 1974; Gilbert & McWeeny 1976; Adachi *et al.* 1979; McWeeny 1979; Walker 1984; Wedzicha *et al.* 1984). It is well known though that sulphites react readily with reducing sugars, carbonyls and proteins of fruit to yield a variety of organic combined sulphites. When sulphur dioxide is absorbed into fruit, it is converted mainly to the bisulphite ion. The bisulphite ion can remain free and available to retard the formation of Maillard-type compounds, such as the carbonyl groups of aldehydes (Gehman & Osman 1954). This bound sulphite is considered to have no retarding effect on product deterioration (Burroughs & Sparks 1973b). With dried fruits, the concentration of absorbed sulphur dioxide is a function of the sulphite concentration in the dip solution, the immersion time and the pH (Stafford *et al.* 1972). To maintain the desired qualities in dried fruit it has been found necessary to incorporate in it an excess of sulphur dioxide to allow for losses occurring during drying, processing and storage.

Our knowledge from the literature concerning sulphuring of figs is from some fairly old published works of Howard (1901), Rixford (1918), Nichols & Christie (1930), Nichols & Reed (1932), Nichols (1933), Abdulov (1938), Long *et al.* (1940), Condit (1947), so treatment conditions have of course changed in the intervening years. Although a considerable amount of work has been carried out on the methods of sulphuring dehydrated fruits and vegetables as well as on the changes in sulphur dioxide content during dehydration and storage (Legault *et al.* 1949) and the relation of sulphite content and sulphite disappearance to changes in

colour, flavour and nutritive value during storage (Mangan & Doak 1949; Gilbert & McWeeny 1976; Wedzicha *et al.* 1984), nothing has been published for figs. In Greece, except some fairly old studies of Kalogereas (1932, 1935) for drying and sterilizing of figs, there is a lack of published information. In recent years, however, little attention has been given in the New World to sulphuring dried fruits because of the tension of eliminating use of any kind of additives in foods ("healthy food"). But as sulphured figs remain a desirable product at the European market, sulphuring is still a method widely adopted for preservation of dried fruits (e.g sultanas, apricots and figs) specially in Greece.

Many methods have been established for the measurement of residual sulphite levels in foods (Nury & Bolin 1965, Davis *et al.* 1983, Beutler 1984, Anderson *et al.* 1986, Sullivan *et al.* 1986, Warner *et al.* 1987, Perfetti *et al.* 1989) but correlations between the various methods have not yet been established for most foods. Also, very little residue data are available for sulphited foods obtained from the market place. Much of the available data were obtained from product samples immediately after processing. Therefore, the effects of storage and any differences with standard, present day commercial practices, have not been taken into account. As mentioned in the literature review (pp 16-28), the effects of treatments before packaging on residual sulphite levels have essentially been ignored in previous work.

The variety of figs used at this study, dried figs of Kymi, Greece, are marketed in packs of 500 g, with a moisture content of 17-25 %. Ripe "Kymi" figs have a yellow-greenish skin which is thin and sensitive to splitting in comparison with other greek cultivars (Papaioannou A., personal communication 1989). That unique thickness of the skin make the figs favourable for sulphuring. The method of sulphuring used by the farmers, is an old one, consisting in burning an amount of elemental sulphur underneath a wooden tray full of figs. Fumes of burning sulphur were considered to be the cheapest source of sulphur dioxide. The figs were first covered with a plastic. Figs being sulphured by that way, were found to

absorb 3 times more sulphur dioxide than the upper limits permitted for foods generally in Greece, which are 750 ppm for fresh and 2000 ppm for dried figs (Greek Food Code 1984). That method, except for its difficulty, has certain other disadvantages, such as the inconstant conditions and the environmental pollution. In addition, the absorbtion and retention of sulphur dioxide is depending on length of burning and the amount of sulphur used. The introduction of melted sulphur into the fermenter either by direct melting and deposition or by volatilization, the introduction of undesirable products of combustion from the supporting cloth, and the difficulties in measuring and regulating the amount of sulphur dioxide introduced, strongly led to limit this method.

It was necessary then to evaluate some other methods of treatment, so that the figs could absorb less sulphur dioxide and at the same time got the desirable pale yellow colour. As noted at the literature review (pp 16-28), a very important factor in technology of dried figs is the constant loss of sulphur dioxide during drying and storage of the product, when its beneficial action is minimised and its levels inside product are allowable. The amount of sulphur dioxide contained in figs plays an important role in the marketing as well as in their storage stability.The actual preservative action of the added SO_2 is contributed to free SO_2, but the level of total SO_2 is also very important as in high levels may be hazardous to human health. Thus it was important to estimate total SO_2 for legislative purposes. It was important to determine also the effect of several factors as sulphuring method, storage conditions (time, composition of the atmosphere, temperature, packaging material etc.) on levels of sulphur dioxide in the figs.

In recent years, there is pressure on the small food manufacturers in several EU countries to adopt specifications for quality and safety standards. In anticipation of the adoption of "new" technologies in Greece, the objectives of this part of the study were: 1) to improve the old method used for sulphuring figs and also develop a new sulphuring procedure of low cost that could be easily adopted by the Greek

farmers, easy to control the amount of sulphur dioxide absorbed and simultaneously result in a product of good quality 2) to study the changes in sulphur dioxide content (free, combined and total) during storage of dried figs under various conditions.

Further studies about the effect of sulphur dioxide on the extension of shelf life of dried figs will be discussed in Chapter 4 of this project. One paper has been published on this work; offprint is presented in the Appendix.

Materials and methods

The materials and methods are given (pp 50-56) in section A of Chapter 2. Concerning the experiments for improving sulphuring procedure, it is worthy to describe the method used for so long by the producers.

1. Figs were picked from the tree when they were ripe enough to be eaten out of hand (colour of figs green to yellow). The fully mature figs that remained on the tree and those in better condition that had fallen down, were dried without sulphuring ("aklivanista").

2. The stems were cut, the figs were then spread in wooden trays and sulphured by burning sulphur. The amount of sulphur powder and the duration of sulphuring is empirically defined by the farmers. After sulphuring, the figs were almost split in two and exposed to sunlight for drying for 2-3 days. The trays are placed on stands (Fig 3.1) because that way the fruit dried faster and had less chance of becoming contaminated with dirt. After drying, the figs were stored for some days at the farmers warehouses before being gathered by the Agricultural Cooperative.

3. At the Cooperative, the figs were fumigated with methyl bromide to control insect infestation which may have occurred during sun drying, following this they were stacked for a short time, rinsed with cold water for 3 seconds and

Fig. 3.1. Sun drying of figs; The sulphured fruits, were split in two and spread in wooden trays that lie on stands.

subsequently washed with hot water at 60-70°C for 20 seconds, then dried again first with hot air at 50°C for 30 sec and then at room temperature overnight and finally packed. The time between sulphuring and packaging of figs was about a month, an important period because a great part of the sulphur dioxide was lost during that time.

As part of the experimental methods, we had also introduced fresh figs cut from the tree only when mature. The figs were then treated with different methods of sulphuring and then dried. The total amount of sulphur dioxide of the product was determined at the time of packaging.

The methods of sulphuring figs that have been studied were:

1) Burning sulphur (powder): the burning of sulphur was done in a closed chamber of dimensions 1.20x1.00x0.60 m using 7 different amounts of sulphur; 200, 400, 600, 800, 1100, 1400 and 1800 g of sulphur. The powder was obtained from the market and its purity has been tested. A good grade of refined sulphur is recommended as being more economical and less trouble than cheaper grades. Special care was taken so that sulphur was burned completely. The amount of fruit that was sulphured each time was of 100 kg weight and was placed in 10 wooden trays, the duration of burning was 2 hours. After sulphuring, the fruits were exposed to sunlight for 3 hours for drying, at an average temperature of 40°C.

2) Spraying the product with sulphur dioxide solutions of various concentrations, from 2 to 6 % w/v in water, while the fruits were exposed to sunlight lying in the trays. The figs were either covered with a plastic film after spraying that allowed sulphur dioxide evaporation, or they were hermetically sealed in plastic film so that sulphur dioxide evaporation was impossible. The amount of fruit sulphured each

time was of 10 kg weight. Sun drying of figs was accomplished by the regular commercial procedure but for 3 hours as in method 1.

3) Dipping figs into sulphur dioxide solutions of various concentrations, ie 2, 4, 6 % w/v in water for 3 different times (5, 10 and 15 minutes). Each time 10 kg of figs were placed on a matrix and were dipped into the solution of sulphur dioxide. They were then put on wooden trays and sun drying was accomplished by using the commercial procedure for 3 hours.

4) Exposing figs to vapour produced from a sulphur dioxide solution of 6 % w/v in water (with 1 litre of solution for each 10 kg of figs). To do this the figs were put on trays in an enclosed chamber made from transparent plastic which was as air tight as possible (Fig. 3.2). The duration of sulphuring was 2 hours. Other figs were put into a similar chamber made from wood, having a sufficiently tight construction to prevent drafts, except of course, that sunlight exposure was impossible (Fig. 3.3). After such sulphuring, the figs were exposed to sunlight for drying as described previously.

5) Direct gas sulphuring by submitting the fruit to sulphur dioxide gas provided by a bomb of sulphur dioxide. The duration of sulphuring was 2 hours at a constant pressure. In one case the figs were in a dark chamber (see Fig. 3.3) where sunlight exposure was impossible. The other lot of figs was put in the transparent chamber (Fig. 3.2) and therefore exposed to sunlight. In both cases, after sulphuring, the fruits were further exposed to sunlight for drying.

A synopsis of the experimental work done in this part of the study is given in Table 3.1. The samples for analyses were secured by random selection in respect to individual pieces, whilst providing that each sample contained pieces from all trays and from all parts of each tray.

Fig. 3.2. Chamber from transparent plastic used for sulphuring figs.

Fig. 3.3. Wooden chamber used for sulphuring figs. The sunlight is excluded.

Table 3.1. The methods of sulphuring used in this study.

For column headings see legend below

(1)	(2)	(3)	(4)	(5)
a	200 g	120 min	yes	yes
	400 g	"	"	"
	600 g	"	"	"
	800 g	"	"	"
	1100 g	"	"	"
	1400 g	"	"	"
	1800 g	"	"	"
b	2%	90 min	yes	yes - no
	4%	"	"	"
	6%	"	"	"
c	2%	5-10-15 min	yes	yes
	4%	"	"	"
	6%	"	"	"
d	6%	120 min	yes	no
	6%	"	no	no
e	nd	120 min	yes	no
	"	"	no	no

(1) Source of sulphur dioxide
(2) Amount of sulphur or % concentration (w/v) of sulphur dioxide solution used
(3) Duration of sulphuring
(4) Exposure to sunlight
(5) evaporation of sulphur dioxide

a: Burn powder of sulphur
b: spray figs with sulphur dioxide solution
c: dip figs into a sulphur dioxide solution
d: expose figs to vapours coming from a sulphur dioxide solution
e: direct gas sulphuring
nd: not determined

Results

Methods of sulphuring

In order to find, as mentioned previously, a more appropriate method for sulphuring figs, the following methods of sulphuring have been compared :

1) spraying figs with a 6% w/v sulphur dioxide solution

2) dipping figs in 6% w/v sulphur dioxide solution for 15 minutes

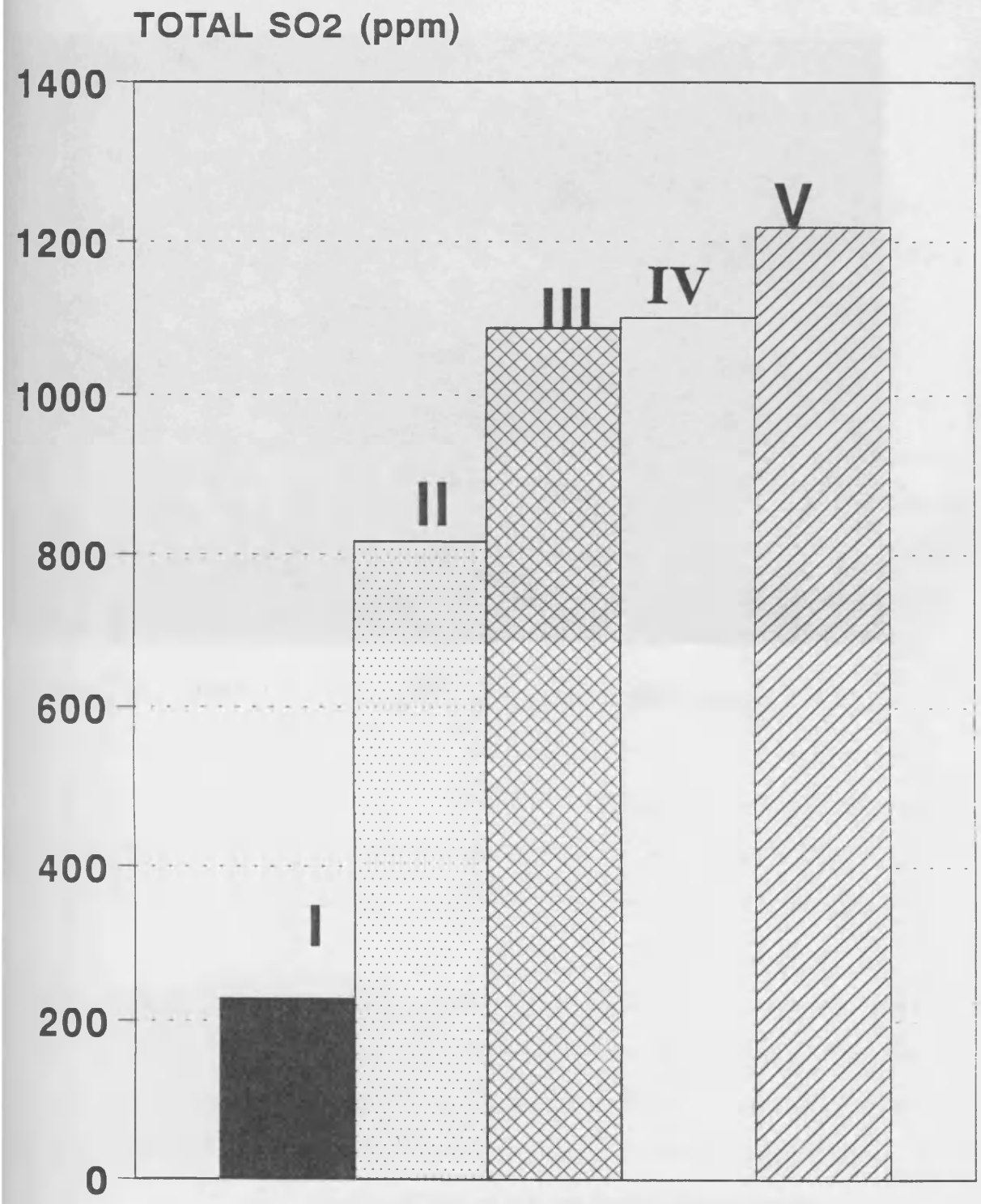
3) direct gas sulphuring for 2 hours from a bomb of gas sulphur dioxide

4) exposing for 2 h to fumes of sulphur dioxide coming from a 6% w/v water solution

5) burning 800g sulphur for 2 hours (procedure usually followed by farmers for the figs of Kymi, till summer of 1990).

As Fig. 3.4 shows, spraying seemed to be the method that resulted in a product with the lower content of sulphur dioxide. However, it has a disadvantage which concerns the intensity and similarity of the colour of the figs. Also, the low initial SO_2 amount may be proved inadequate for acting as a preservative during storage. Of interest though, is the method (IV) of exposure to sulphur dioxide fumes produced from a sulphur dioxide solution for the following reasons: it is easy, quick (demands 1-2 hours), the figs obtain a satisfactory similar yellowish colour and most of all, it is possible for farmers to control the amount of sulphur dioxide absorbed by the figs, by adjusting the concentration or the volume of sulphur dioxide solution. The time of exposure of the figs to this sulphur dioxide is also easy to be controlled. Special attention must be paid to the role that sun irradiation plays in the formation of the colour of the product. Figs that have been sulphured according to method (IV) in a chamber made from a transparent plastic (see Fig. 3.2) allow only exposure to sunlight to figs on the upper pallet. Therefore this pallet of figs develop the desirable colour quickly (Fig. 3.5a). On the other hand,

Figure 3.4:Influence of method of sulphuring on sulphur dioxide content of dried figs



(I) : spray with 6% w/v SO_2 solution
(II) : dip into a 6% w/v SO_2 solution
(III): direct gas sulphuring
(IV): fumes from a 6% w/v SO_2 solution
(V) : burn 800 g S (traditional method)

Fig 3.5. Figs sulphured by fumes of sulphur dioxide solution in a "transparent" chamber. Only the figs at the upper pallete (a) got the desirable colour after sulphuring.

(a) (b) (c)

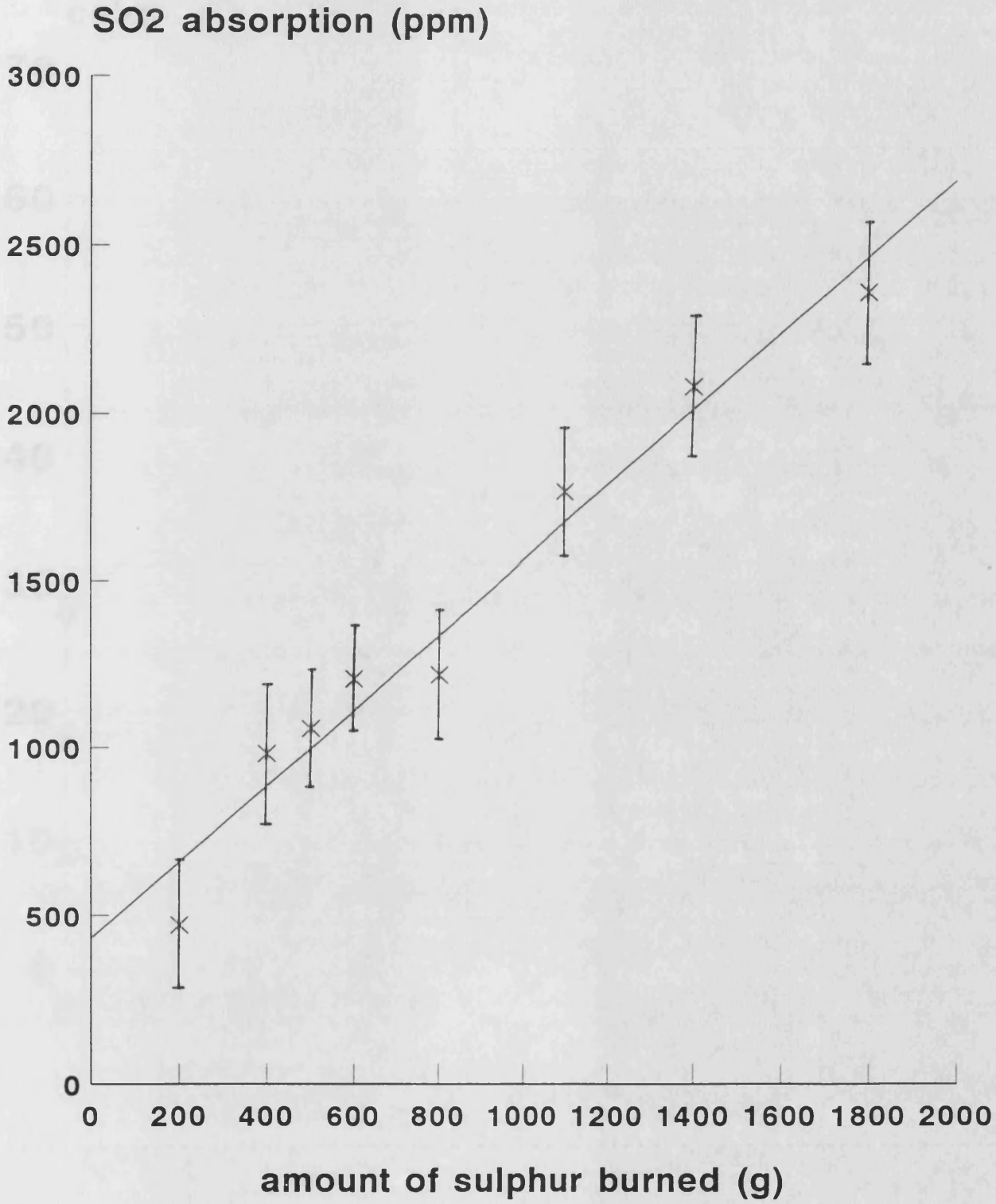
figs on lower pallets (Fig. 3.5b,c) along with those that were sulphured in a dark chamber (Fig. 3.3) have not developed their colour (Fig. 3.6a,b) and therefore need to be exposed to sunlight for at least 1-2 hours after sulphuring, to obtain their desired colour (Fig 3.6c).

With reference to the traditional method of sulphuring, our studies stress the profound influence of the amount of sulphur burned on the total sulphur dioxide absorbed by the figs. A progressive increase in the total sulphur dioxide absorbed by the figs was observed when larger amounts of sulphur were burned in the chamber (Fig. 3.7). There seemed to be a linear correlation between the two parameters (R=0.9827). It is evident from Fig. 3.7 that the optimum amount of sulphur needed to keep the final sulphur dioxide content below the 750 ppm limit is a burn of 400 g of sulphur powder. The amount of sulphur being used during the sulphuring procedure has also an effect on the colour of the figs. As can be seen from Fig. 3.8, there is a gradual increase of the "L" parameter, the brightness of colour; and a small decrease of the positive "a" values (deviation from red colour). The "b" parameter was not found to change significantly which means there is no change of the yellowness. On the other hand it is clear from Fig. 3.9 that there is a linear relationship between the "L" parameter of colour (brightness) and the total sulphur dioxide content of figs (R=0.9919). The more sulphur dioxide the figs contain, the more bright they are.

When figs are sulphured by dipping them in a sulphur dioxide solution, their sulphur dioxide content is affected by the concentration of the dip solution. The results obtained (Fig. 3.10) showed an overall trend of increasing sulphur dioxide content of figs with percent concentration of bisulphite solution, the smallest increase being associated with the lower immersion time. These results are similar as those of Stafford *et al.* (1972) for sulphured apricots.

Fig 3.6. Figs sulphured in a dark chamber by fumes of sulphur dioxide solution
(a) before sulphuring procedure
(b) after sulphuring
(c) after being exposed to sunlight.

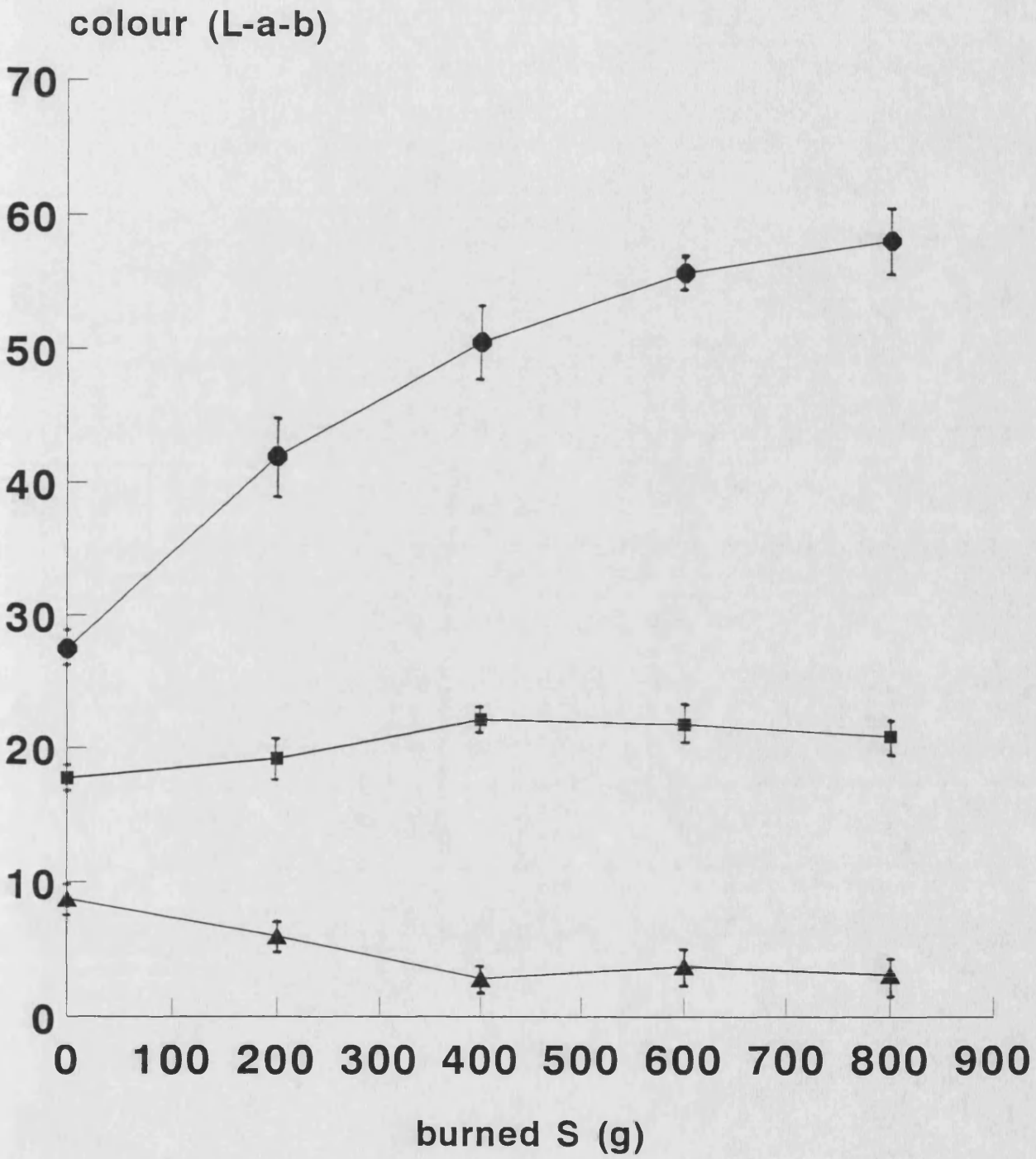
Figure 3.7: Influence of the amount of burned sulphur on sulphur dioxide absorbed by figs



Each point is the average of thirty determinations

The statistics were made as described in materials and methods pp.68

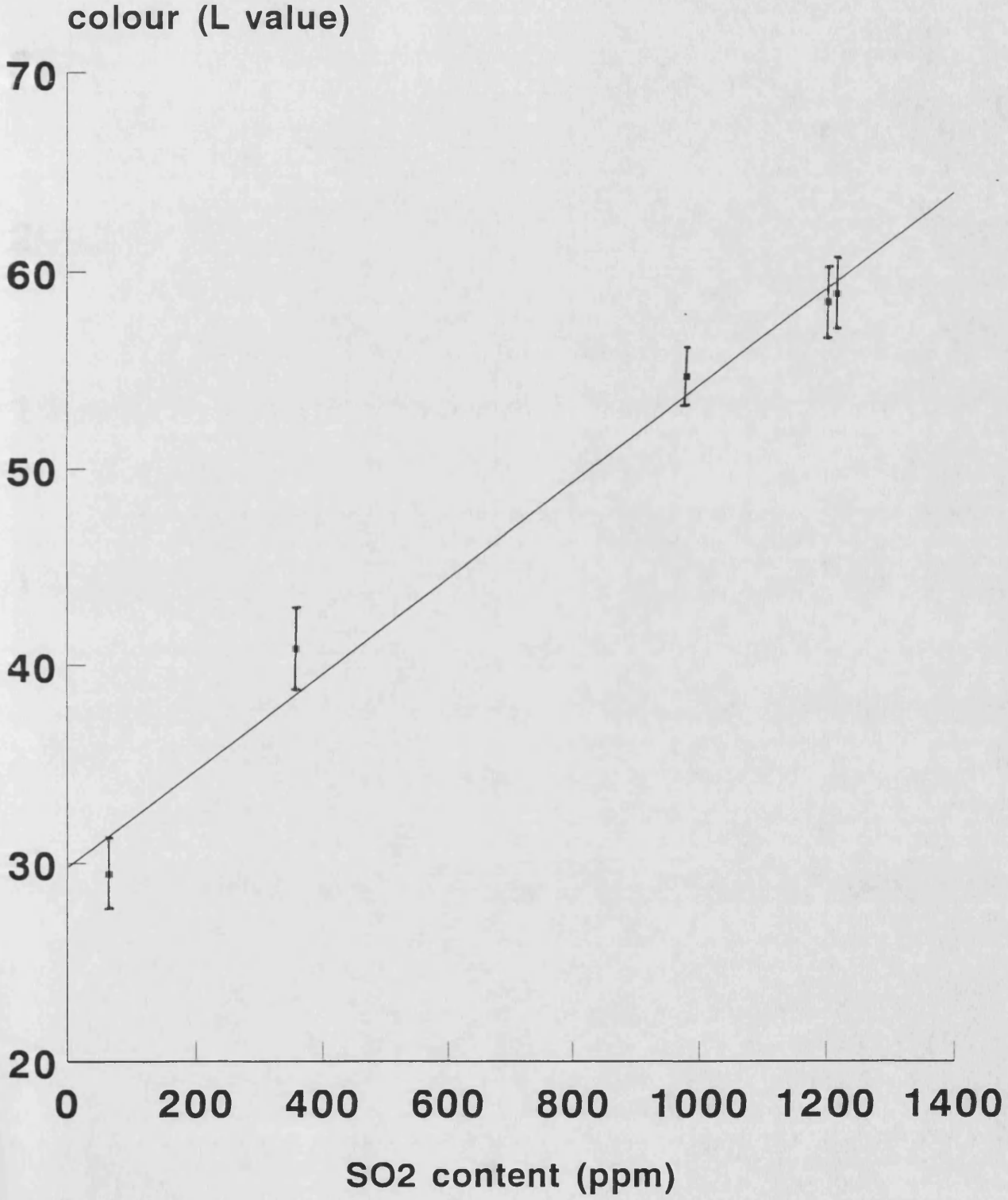
Figure 3.8:Influence of amount of burned sulphur on the colour of figs



Each point is the average of ten measurements

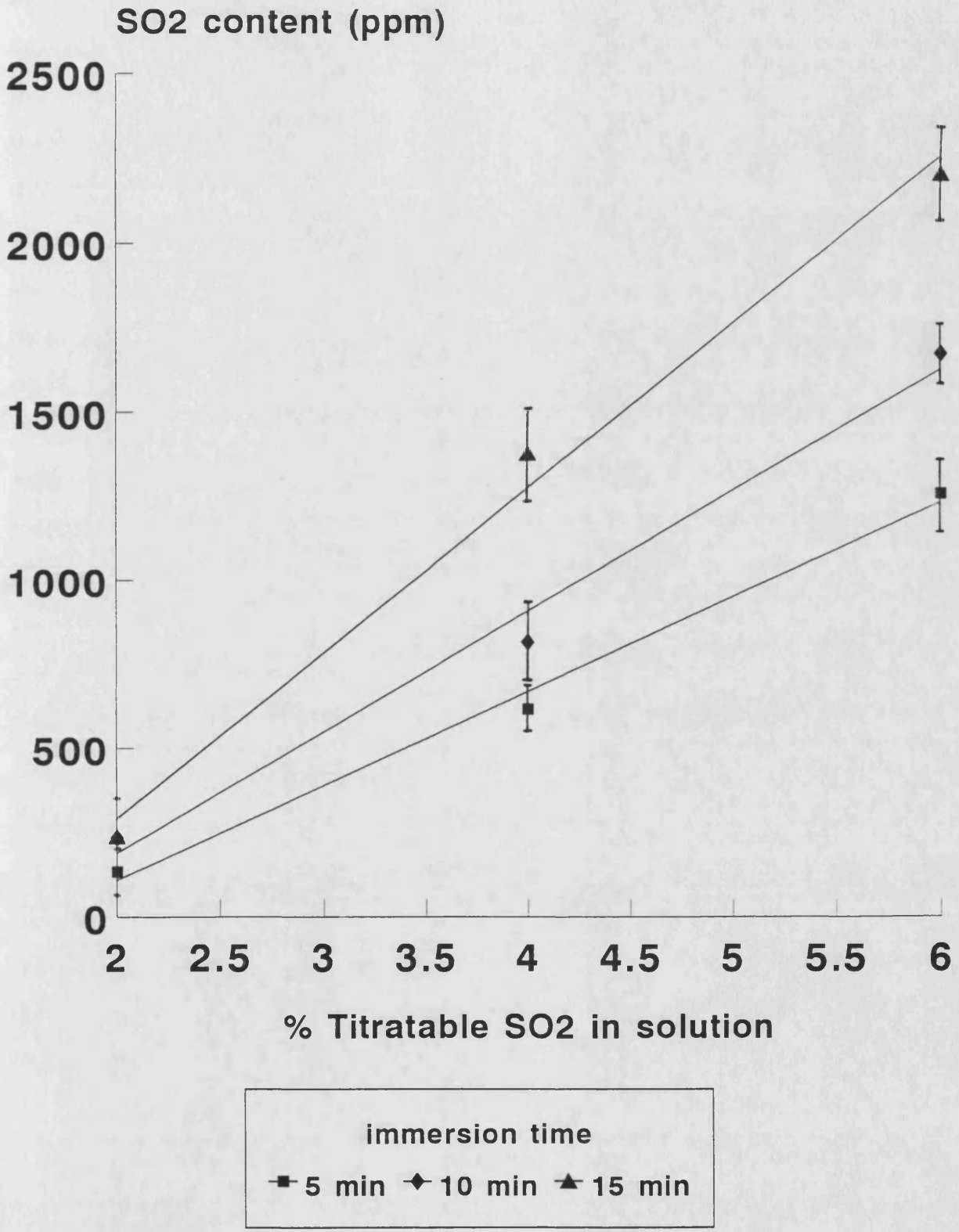
The statistics were made as described in materials and methods pp.68

Figure 3.9:Relation between colour of figs and their sulphur dioxide content



Each point is the average of five measurements
The statistics were made as described in materials and methods pp.68

Figure 3.10:Effect of concentration of the dip solution on the sulphur dioxide content of bisulphite treated figs

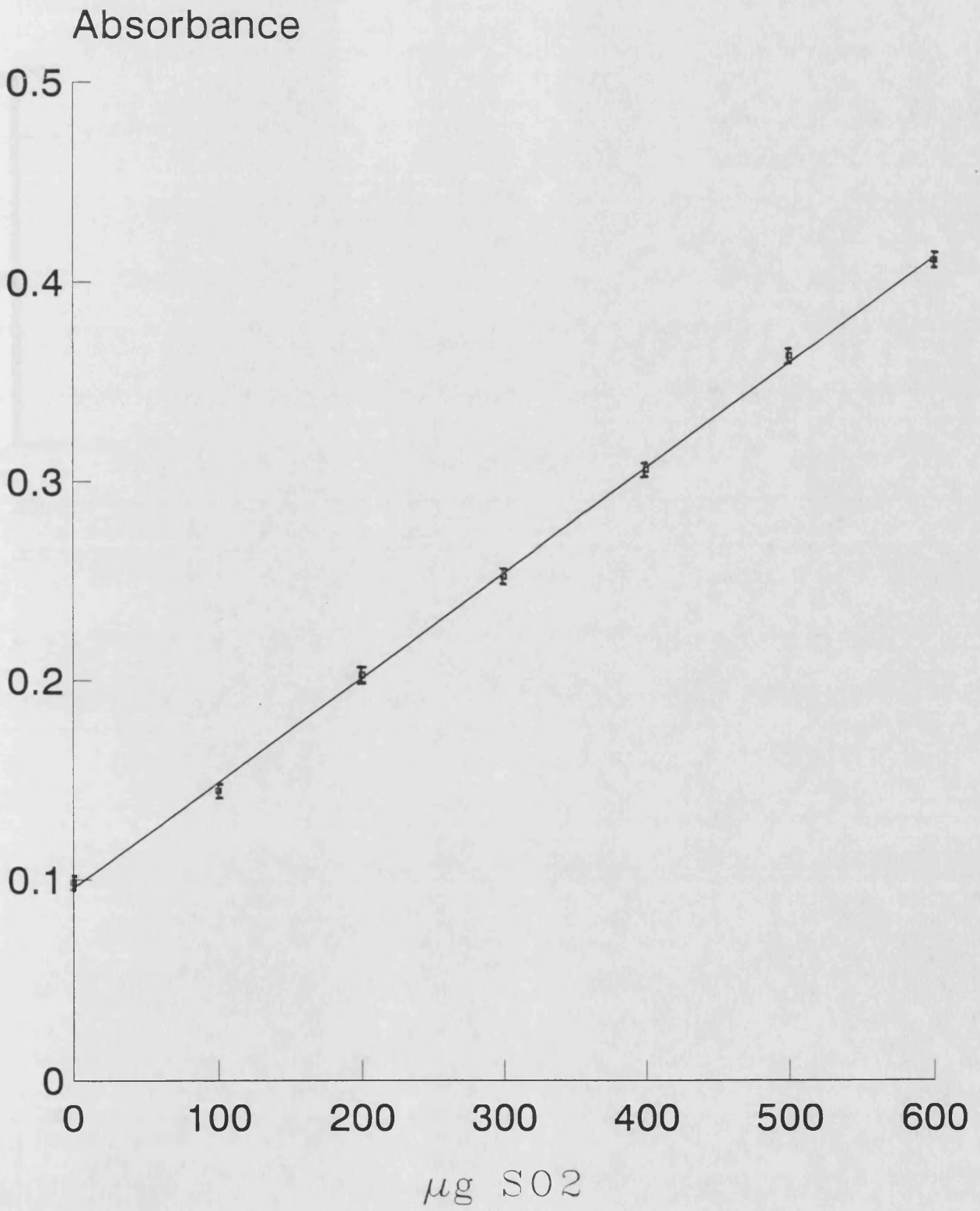


Each point is the average of five determinations

The statistics were made as described in materials and methods pp.68

At this point in the study, it is noteworthy to report a comparative study that has been made in order to stress the superiority of the Monier-Williams distillation method over the colorimetric method of p-hydrochloric rosaniline in samples containing large amounts of SO_2. A standard curve has been drawn by measuring the absorbance at 550 nm of seven standard solutions of sulphur dioxide containing 0-600 μg SO_2 (Figure 3.11). A series of samples of sulphured dried figs with six different SO_2 levels were analyzed with both the distillation and colorimetric methods for total sulphur dioxide content. The mean values of the ppm content of the samples are given at Table 3.2. As can be seen, only at high SO_2 levels the mean values obtained by the colorimetric method were significantly lower ($P<0.05$ or $P<0.01$) than values obtained by the volumetric Monier-Williams method. A "t-test" was used to show the differences in means. The variances of the methods were, in fact not significantly different ($P<0.02$). The recoveries of the two methods are very satisfactory (average 87 % for the Monier-Williams and 97 % for the colorimetric), thus excluding this being the reason of the difference between the two methods. The results presented are in agreement with those published by several workers (Brekke 1963; Nury & Bolin 1965; Suzuki 1977) although from our experiments a greater difference was noticed between the mean values obtained from two methods (average 22 % lower results of the colorimetric method). That difference is maybe due to the smaller sample used (10 g of figs for the colorimetric method instead of 25 g for the Monier-Williams). It should be pointed out though that at levels near those examined by other workers the same differences (about 6 % lower) were found. Although the colorimetric method is less time consuming as a lot of samples can be analyzed in the same time, it is not recommended for samples of high sulphur dioxide levels.

Fig. 3.11:Standard curve of the colorimetric method for determining total sulphur dioxide



The statistics were made as described in materials and methods pp.68

Table 3.2. Comparison results (means and standard deviations) of distillation and colorimetric methods for estimation of total sulphur dioxide content (in ppm) of dried figs.

Sample No	Monier-Williams method (ppm)	Colorimetric method (ppm)
1	294±64	187±48
2	930±101	568±73
3	1205±155	933±103
4	1256±179	1008±67
5	1340±118	1301[a]±94
6	1848±189	1726[b]±146

25 g and 10 g of grounded figs were used for the analyses respectively for both the distillation and the colorimetric method (five replicants in each case)

[a]: significantly lower ($P<0.01$) than Monier-Williams method

[b]: significantly lower ($P<0.05$) than Monier-Williams method

Sulphur dioxide binding and losses

In understanding the sulphur operation, it is clear that only a little of the absorbed sulphur dioxide is bound in the initial sulphuring (McBean 1967). During sun-drying, more of the sulphur dioxide becomes bound and some is totally lost from the system by vaporization and by combination with oxygen to form sulphate. Our studies showed that in dried figs, with an initial amount of total SO_2 of 1950 ppm, 83.6 % of the SO_2 is in bound form. The treatment that figs are subjected before packaging is the hot washing (see p 74). The effect of that "washing" on sulphur dioxide loss is presented at Fig. 3.12. The figs lose about 50 % of their sulphur dioxide content but gain humidity from 18.53 % to 21.73 %.

In order to study the effect of the packaging material on the loss of total sulphur dioxide of figs, three packaging materials were used: the traditional paper box and two plastic films A and B (described in materials and methods p.48). The selection of the two plastic films for packaging had two purposes: a) to use a film highly barrier to water vapour so that figs can retain much of their initial humidity for as long as possible and b) to study how films with 2 different permeabilities to low molecule gases (with main target the SO_2) affect the loss of SO_2 through storage. The loss of sulphur dioxide during a year was monitored (Fig. 3.13). It is evident that the figs packed in highly gas permeable polyethylene, had lost at the end of storage, 60% of their initial sulphur dioxide, while figs in lower gas permeable polyethylene-polypropylene laminate had lost, during the same time, 15% of their initial sulphur dioxide. On the other hand the figs in paper box show a similar dramatic loss of the sulphur dioxide content to the highly gas permeable polyethylene. These results are similar to the findings of Davies *et al.* (1973) for dried apricots.

Fig. 3.12:Effect of hot washing on total sulphur dioxide and humidity content of dried figs

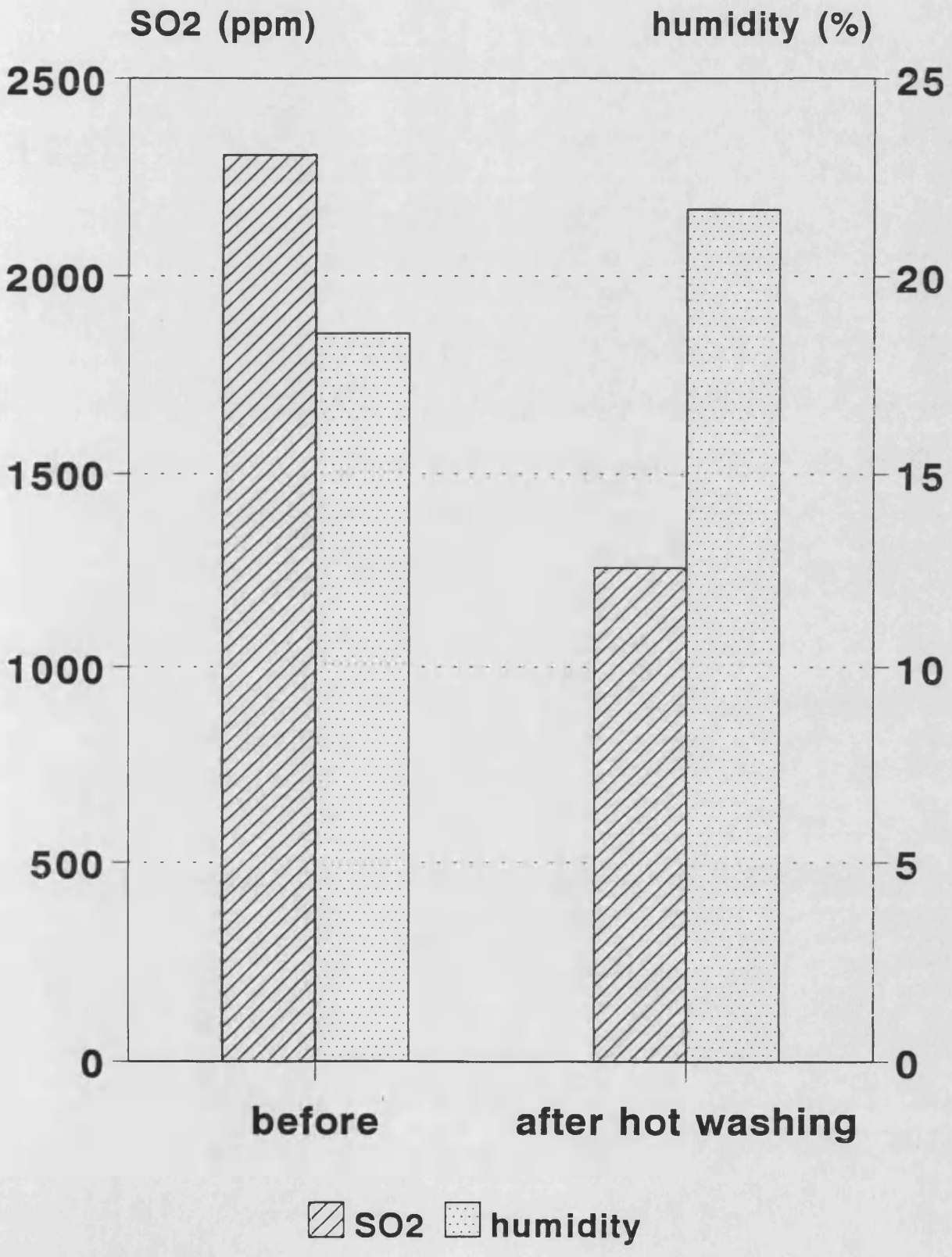
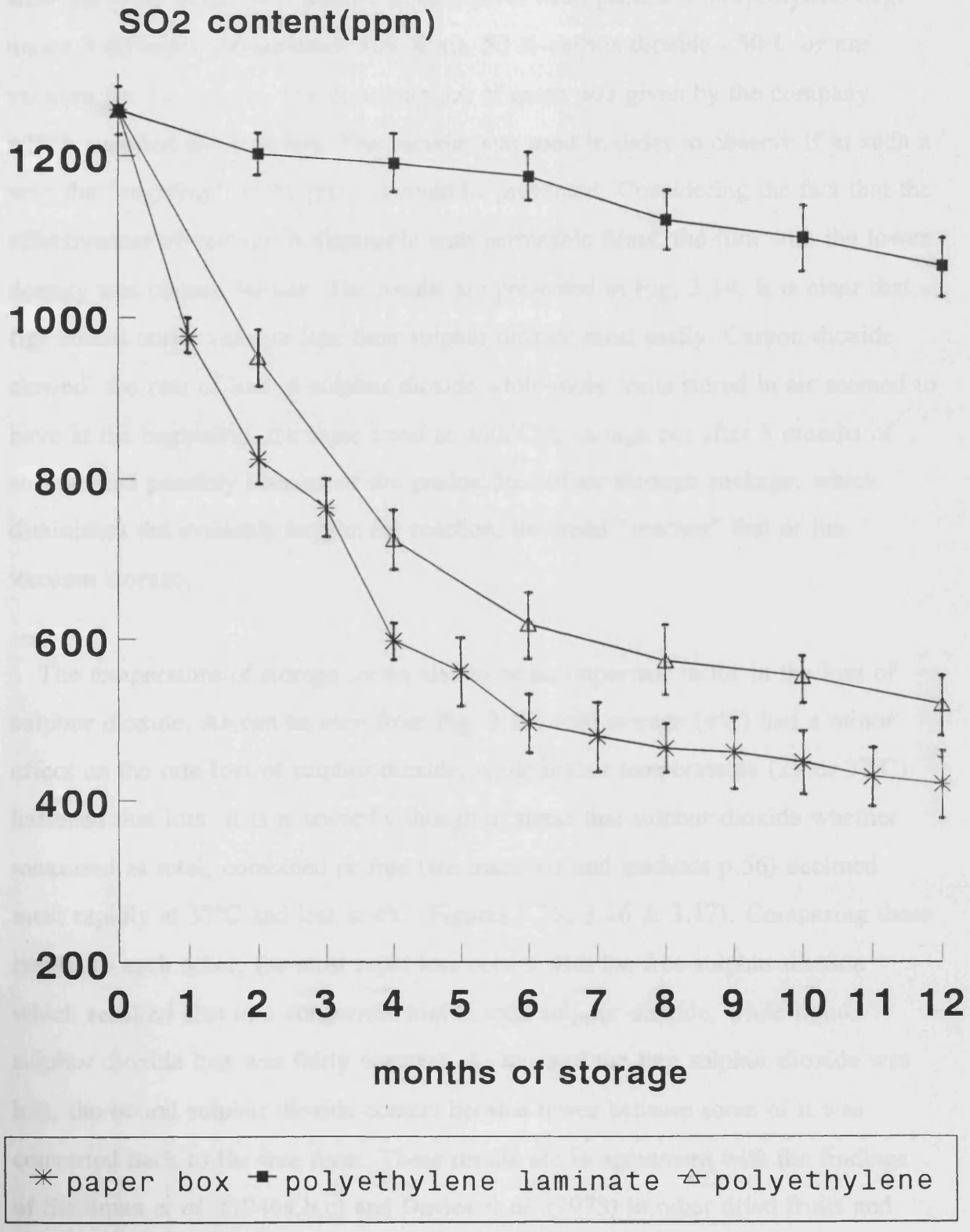


Figure 3.13:Effect of packaging material on loss of total sulphur dioxide absorbed from dried figs in 25 °C



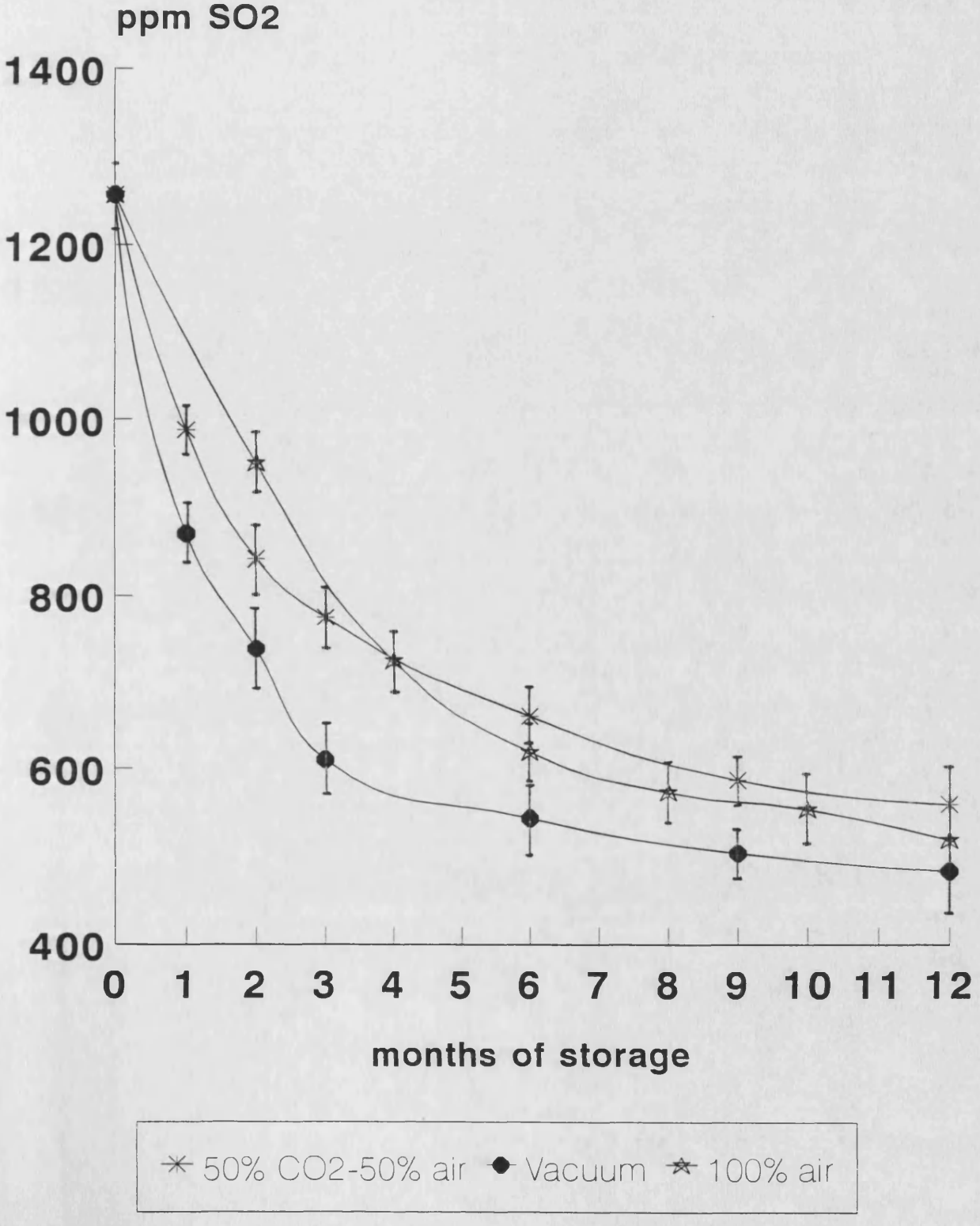
Each point is the average of three determinations

The statistics were made as described in materials and methods pp.68

The composition of the surrounding atmosphere during storage seems also to affect the rate of loss of sulphur dioxide. In order to assess that difference, figs with the same initial total sulphur dioxide level were packed in polyethylene bags under 3 different atmospheres: 100 % air, 50 % carbon dioxide - 50% air and vacuum for 12 months. The concentration of gases was given by the company which supplied the canisters. The vacuum was used in order to observe if in such a way the "sugaring" of the product could be prevented. Considering the fact that the effectiveness of vacuum is disputable with permeable films, the film with the lower density was chosen for use. The results are presented in Fig. 3.14. It is clear that figs stored under vacuum lose their sulphur dioxide most easily. Carbon dioxide slowed the rate of loss of sulphur dioxide while those fruits stored in air seemed to have at the beginning, the same trend as with CO_2-storage but after 3 months of storage and possibly because of the gradual loss of air through package, which diminishes the available oxygen for reaction, the trend "reaches" that of the vacuum storage.

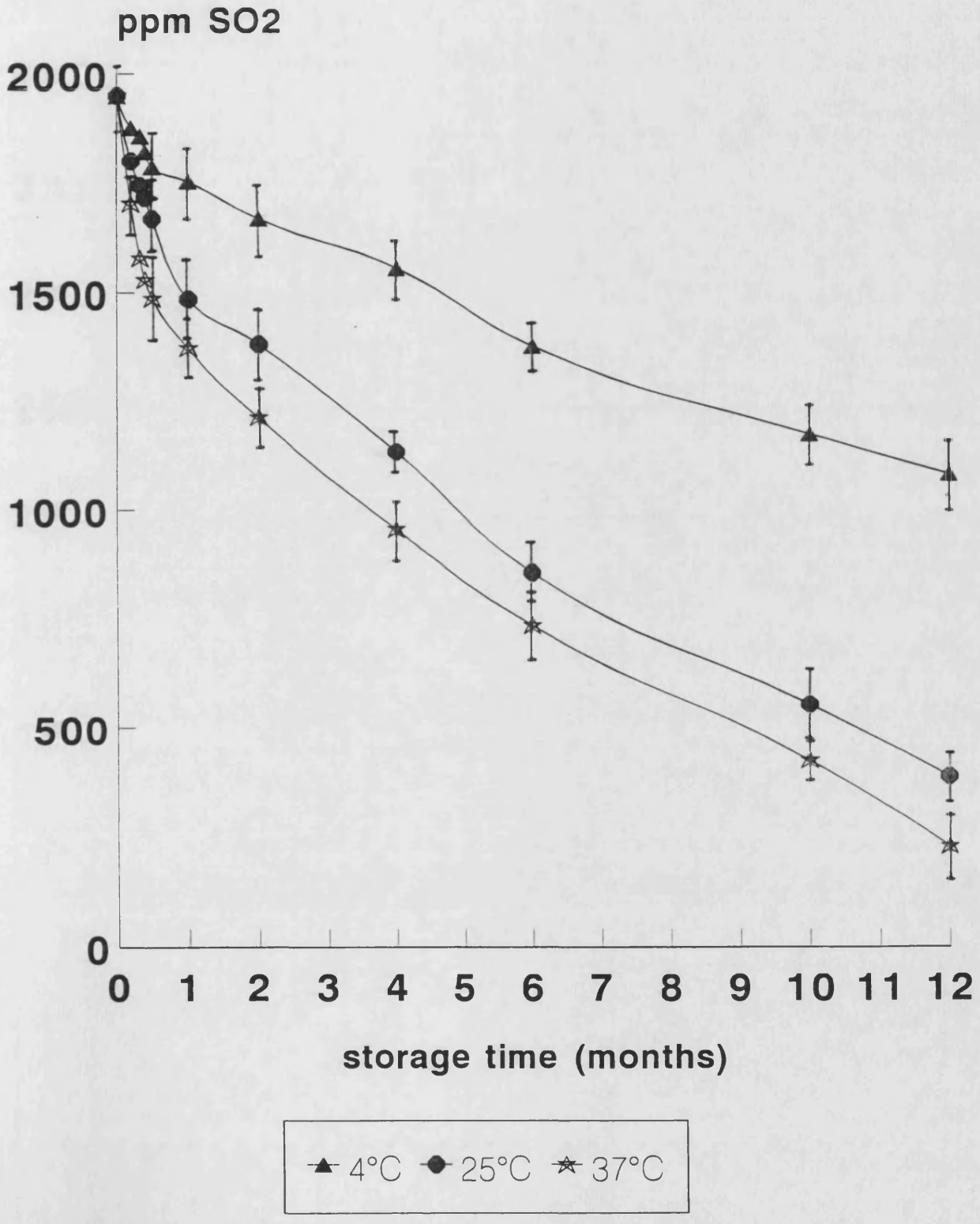
The temperature of storage seems also to be an important factor in the loss of sulphur dioxide. As can be seen from Fig. 3.15, cold storage (4°C) had a minor effect on the rate loss of sulphur dioxide, while higher temperatures (25 or 37°C) hastened that loss. It is noteworthy though to stress that sulphur dioxide whether measured as total, combined or free (see materials and methods p.56) declined most rapidly at 37°C and less at 4°C (Figures 3.15, 3.16 & 3.17). Comparing these results to each other, the most rapid loss occurs with the free sulphur dioxide which resulted also in a concurrent loss in total sulphur dioxide, while bound sulphur dioxide loss was fairly constant. As more of the free sulphur dioxide was lost, the bound sulphur dioxide content became lower because some of it was converted back to the free form. These results are in agreement with the findings of Stadtman *et al.* (1946a,b,c) and Davies *et al.* (1973) in other dried fruits and

Fig. 3.14:Effect of the composition of storage atmosphere on the loss of total SO2 absorbed from dried figs at 25°C



The package used is polyethylene
Each point is the average of five determinations
The statistics were made as described in materials and methods pp.68

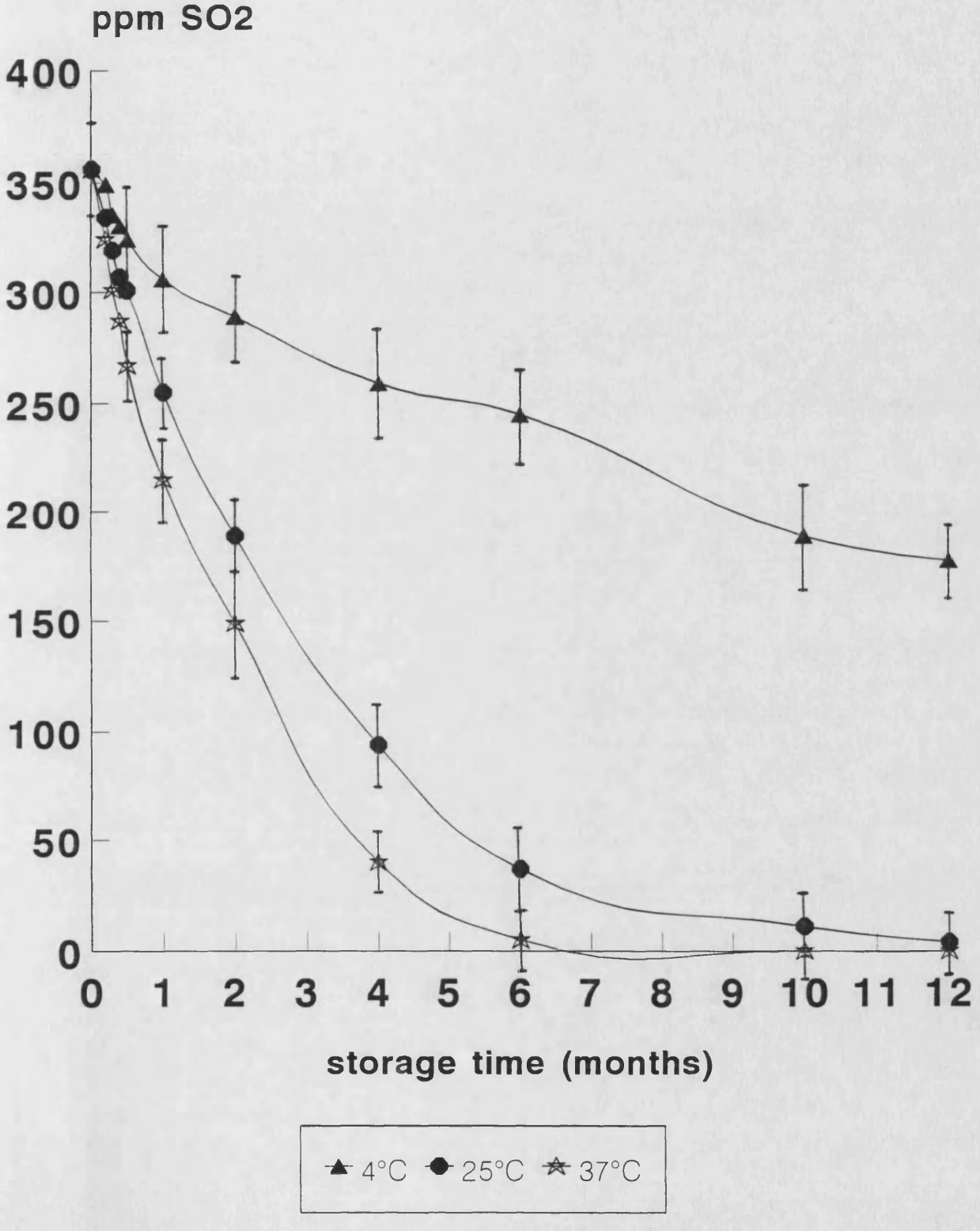
Fig. 3.15:Effect of temperature of storage on loss of total sulphur dioxide absorbed from dried figs



The package used is polyethylene bags under air
Each point is the average of five determinations

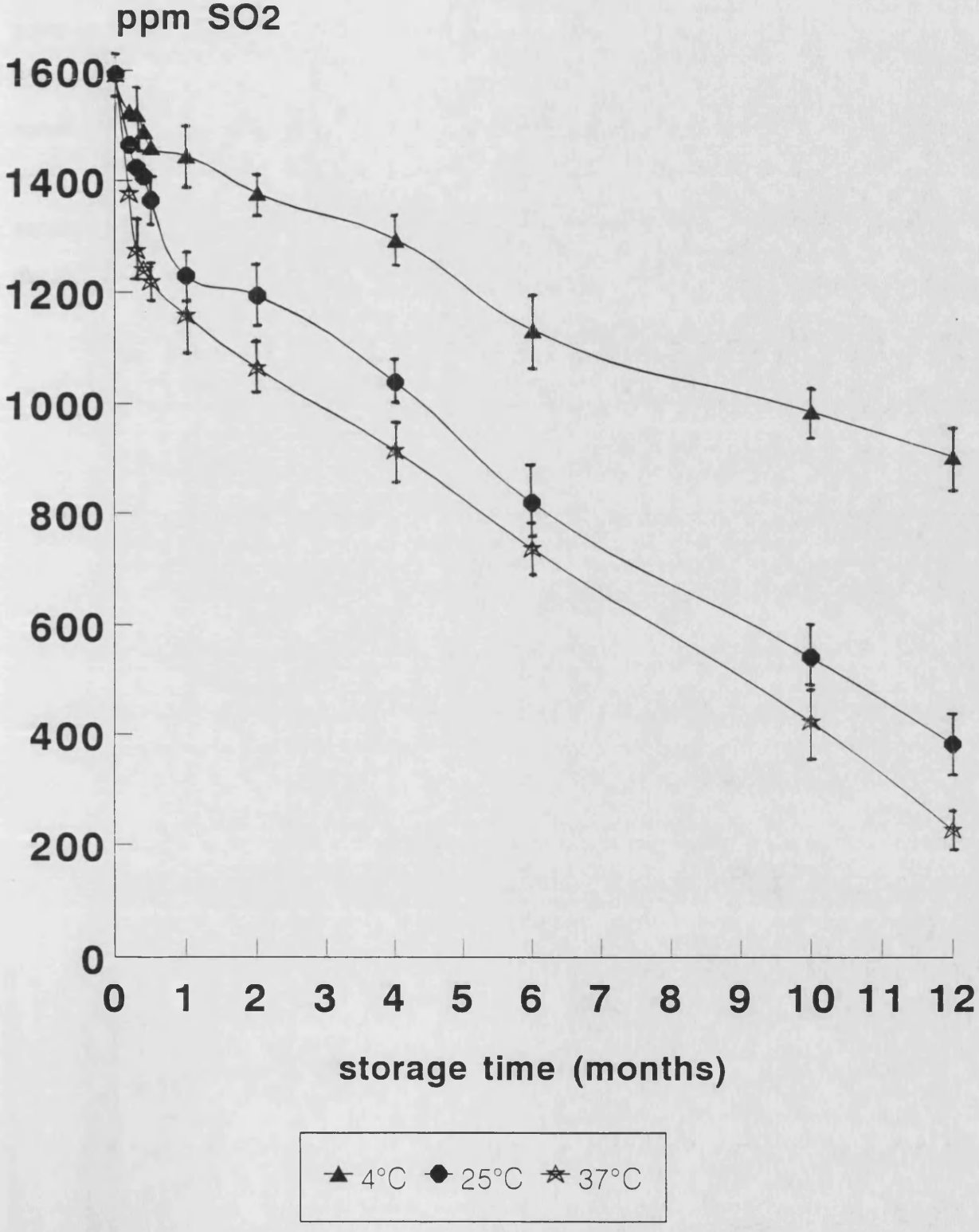
The statistics were made as described in materials and methods pp.68

Fig. 3.16:Effect of temperature of storage on loss of free sulphur dioxide absorbed from dried figs



The package used is polyethylene bags under air
Each point is the average of five determinations
The statistics were made as described in materials and methods pp.68

Fig. 3.17:Effect of temperature of storage on loss of bound sulphur dioxide absorbed from dried figs



The package used is polyethylene bags under air
Each point is the average of five determinations
The statistics were made as described in materials and methods pp.68

Bolin & Jackson (1985) and also the trends are similar to those found by Banks & Board (1982) for smaller amounts of SO_2. From Figures 3.15-3.17 it is also evident that storage temperature affects the binding of SO_2. Cold storage (4°C) prevented any significant binding but when the storage temperature was raised to 25°C, the degree of binding was increased. Storage of the dried figs at 37°C resulted in a further increase of binding. This could possibly be due to the activation energy of the binding reaction because of the higher temperature. It seems that the increase in bound SO_2 is both a reflection of the loss of free SO_2 in the beginning of storage and also effect on degree of binding.

Discussion

The preservative action of sulphur dioxide in food and vegetable products has been known for a long time (Abdulov 1938, Tanner 1944, Woodroof & Cecil 1945, Cruess 1948, Schelhorn 1951, Green 1976). It was evident in this study also. Large-scale experiments in sulphuring dried cut fruits were made by many workers (Chace *et al.* 1930, 1933; Fisher *et al.* 1942; Mrak *et al.* 1942b) but all of them used either burning sulphur or direct gas sulphuring.

In Greece, the method used so long for sulphuring figs by burning sulphur had many disadvantages, the main of which being the inconstant and uncontrolled conditions. A new improved method for sulphuring figs of Kymi has been developed, which provides the possibility of controlling the conditions of sulphuring, thus controlling the quality of final product, is easy to be used by unexperienced people, moneysaving and gives satisfactory results in final colour of the product. The effect of type of sulphur dioxide compound used and the method of application on the changes occurring in colour and flavor during subsequent treatment and storage of food is still largely unknown. Such investigations as have been made have been limited by unavailability of methods of analysis which would determine the distribution of sulphurous acid in the product. It was believed at one time that the concentration of sulphur dioxide improved penetration and retention, but later, Long *et al.* (1940) found that temperature and concentration of sulphur dioxide are the most important factors influencing absorption and retention.

Our studies demonstrated clearly that the amount of sulphiting agent used for sulphuring figs influences the SO_2 absorbed by figs as well as their colour (Figures 3.7, 3.8). These results agree with those reported by Jewell (1927). The fact that this variety has a thin skin means that SO_2 can be easily absorbed but can also be easily desorbed so, it is necessary for the SO_2 to penetrate the fruit tissue

completely and to be well retained. That's why the sulphuring methods of spraying or dipping were not proved so effective.

One of the mechanisms of action of sulphites in fruit discoloration is probably the polyphenol-oxidase catalyzation (Joslyn & Braverman 1954, Taylor *et al.* 1986). By increasing the concentration of sulphiting agent, the amount of the enzyme inactivation is increased, specially in the skin area of the fruit, which affects its lightness (L value, Fig. 3.9). Another mechanism is the reaction of SO_2 with carbonyls of fruit, preventing so the reaction of amino-groups to carbonyls which cause the non enzymic browning (Burton *et al.* 1963, McWeeny *et al.* 1974, McWeeny 1981). The particular characteristic of the variety (i.e. the thin skin) seems to be here also a reason.

Stafford *et al.* (1972) found that the amount of SO_2 absorbed by apricots is a function of concentration of the dip solution, immersion time and pH. This was confirmed in my study as well (see Fig. 3.10). The processing treatments after sulphuring seemed to affect also the retention of SO_2 by the figs. In contrast to the findings of McBean *et al.* (1965) who observed higher SO_2 retentions, mainly in apricots, when fruit was blanched before or after sulphuring and in fruit sprayed with water after sulphuring, our experiments demonstrated a significant decrease in retention of SO_2 after hot washing (Fig. 3.12). This could be due to the fact that figs are already dried, so there are little possibilities of plasmolysis and SO_2 absorption of fruit.

In the present study, about 80-85% of the SO_2 absorbed by figs was found in the bound form in the final dried product depending on the initial SO_2 absorbed. That percentage is higher than the findings of Bolin and Jackson (1985) in dried apricots and apples and near to the 55-80% percentage found by McBean (1967) in dried apricots. Possible explanations for the lack of agreement could be the use of

an experimental sulphuring chamber, where the fruit was exposed to a constant high concentration of SO_2 during the entire sulphuring time. In the old traditional method of sulphuring, the gas was produced for only a short initial period peaking for a few minutes after which it dropped off, ending at a lower concentration.

The retention of SO_2 by fruits during sulphuring and drying has been reviewed by several authors (Nichols *et al.* 1938, Fisher *et al.* 1942, McBean *et al.* 1963, McBean *et al.* 1965). While drying stabilizes the amount of SO_2 held in the fruit tissue, a further slow loss of the preservative occurs during its storage life, which may be up to 12 months. As many authors pointed out (Gilbert & McWeeny 1976, McWeeny *et al.* 1980, Wedzicha *et al.* 1984), SO_2 retained by fruit is lost through storage in a number of ways: (1) it can be physically lost as SO_2 if the pH of product drops below pH 4.0 (2) many of the sulphites in nonacid products can be converted into combined sulphites, mainly complexes with sugars, carbonyls or aminoacids (3) some of this combined sulphites are very stable and cannot be recovered by conventional methods of analysis (4) oxidation of sulphite to sulphate by oxygen (Stadtman *et al.* 1946b, Davies *et al.* 1973). Stadtman *et al.* (1946 a,b,c) in their studies of the factors which influence the rate of darkening of dried apricots during storage, showed the importance of SO_2 level, moisture content, temperature and oxygen availability. Most of their experiments were done under carefully controlled laboratory conditions but at high temperatures (40-50 °C). Nury *et al.* (1960) did similar experiments with dried apricots with storage temperatures ranged from 1.3-32.2 °C. Both the above groups of investigation measured total SO_2 but Nury *et al.* (1960) when showing that rate of loss was higher from samples with higher initial levels, proposed that this may have been due to the presence of larger amounts of free SO_2. The present study is the first attempt to define the rate of loss of SO_2 from figs during prolonged storage under a variety of conditions and packaging. It is clear that this study has given similar results to those obtained for other fruits (Nichols & Reed 1931, Davies *et al.* 1973, Sayavedra & Montgomery 1983). It is probable that most of total SO_2 is lost from figs during the 12 months of storage. It was also found that the processing treatments after

sulphuring and before packaging lowered the amount of SO_2 retained by the figs at such a level that is allowable by Food Code regulations and also is enough to produce a safe storage. The figs packed in paper packaging which is usually used for retail market, showed a dramatic loss of total SO_2 content as well as of humidity which consequently resulted in a product of inferior quality (Fig. 3.13). On the other hand, we have found that an appropriate plastic film package of polypropylene-polyethylene laminate with low gas permeability is a satisfactory alternative for preventing SO_2 loss. Further studies ought to look at the portion of SO_2 which is converted to sulphate in that plastic packaging. At the moment this can not be measured. It may be that during storage, the composition of the gas phase within the plastic bag changes, possibly due to diffusion of oxygen. As O_2 availability to the system is a major factor that influences SO_2 binding (due to oxidation) the experiments done in the polyethylene bags under 3 different atmospheres, suggested that CO_2-enriched atmospheres and to a lesser extent, air are the most appropriate storage atmospheres for sulphured dried figs. These results agree with the ones reported by Salunkhe & Desai (1984) for fresh figs. Also as will be seen later (Chapter 4-5), CO_2-enriched atmospheres, provide a protection in dried fruits against microbial infection. Our studies also confirmed that higher temperatures of storage accelerate the rate of SO_2 loss and binding (Fig. 3.15, 3.16, 3.17). An appropriate temperature of storage would be around 20-25 °C. Lower temperatures are more effective but not applicable, as in retail market shelves the temperatures are around 25 °C.

A general remark for all the curves of sulphur dioxide loss through storage is that the rate of loss is slowed through time. Also a common observation is that the SO_2 declines until about 65% is lost at the end of storage, which agrees with the findings of Stadtman *et al.* (1946b).

CHAPTER 4
Studies of physicochemical and microbiological changes that occur during commercial and cold storage of sulphured dried figs

Introduction

Fruits for drying are subjected to microbial contamination at various stages during development and processing (Frazier 1967). Growth of some of these organisms may take place before the fruits reach the processing plant, if environmental conditions permit and it may continue in the plant up to time of drying, when equipment and workers may contaminate the product. Phaff *et al.* (1946) first have observed that during sun-drying the yeasts present on fresh fruits were not killed and may temporarily increase during the process. According to Natarayan *et al.* (1948), yeast population may be increased greatly during sun-drying under unfavourable weather conditions. Although drying destroys some microorganisms bacterial endospores survive, as do many gram-negative and positive bacteria. Some of the pretreatments may reduce numbers of organisms and others may increase them. After packing, chances of microbial contamination are very low, as the products are usually packed in containers impermeable to microorganisms. Fungi capable of producing toxins that were present in the products before packing, however, will be transferred to the consumer via this route.

A common feature of dried fruits is that a low water activity (a_w) is the basis for their preservation. When spoilage of these commodities occurs, the same species of osmophilic yeasts and xerophilic fungi often are responsible. Fungal spoilage of dried fruit generally does not occur when the water content is under 25%, because at this level the a_w is too low to support growth of most yeasts and fungi (Beuchat 1987). The figs may undergo spoilage while still on the tree prior to drying. Yeasts, introduced into the interior of the fruit by insects, ferment the juice of the florets that line the inner walls. This type of spoilage has been referred to as "souring" because the juice that drips from fermented fruit has a vinegar-like odour

presumably because of subsequent oxidation of ethanol by acetic acid bacteria (Mrak *et al.* 1942a). According to Miller & Mrak (1953), *Acetobacter* was associated with dried fruit beetle *Carpophilus hemipterous*. Experiments with acetic acid bacteria isolated from figs showed though that they alone produce only traces of volatile acid which cannot account for the acidification observed in fig spoilage. It is concluded though that most of the fig souring apparently results from the associative action of yeasts and acetic acid bacteria.

The antimicrobial action of sulphur dioxide is well documented, although the exact mechanism is not known (Cruess 1912; Schelhorn 1951; Rhem 1964; Schroeter 1966; Ough 1983). It is stated generally that SO_2 acts as both a biocidal and biostatic agent on microorganisms in the following order of effectiveness: bacteria > mould and yeasts, and Gram-negative bacteria > Gram-positive bacteria (Hammond & Carr 1976; Lueck 1980). When considering the antimicrobial activity of SO_2 and its salts, three main groups of microbes are of interest in the more acid fruits. These are: (1) acetic acid-producing and malo-lactic bacteria (2) fermentation and spoilage yeasts and (3) fruit moulds. Cruess (1912) found that 310.000 viable *Acetobacter* cells/ml exposed to 100 and 200 mg/l (ppm) total SO_2 were reduced to 300 and 2 cells/ml respectively, after 36 h. Another report of bactericidal action on *Acetobacter* listed 200 mg/l as the necessary concentration for killing activity at a pH of 6.0 (Rhem & Whittman 1962). Dupuy & Mangenet (1963) noted that even small doses of SO_2 inhibited the activity of the cells, but much larger doses were required for cidal action. Yeasts are intermediate to acetic and lactic acid bacteria and moulds in their sensitivity to SO_2 and the more strongly aerobic species are generally more sensitive than the more fermentative species. Investigations of the antimicrobial action of sulphurous acid are difficult to make, as there exist a great number of factors with influence on this action preventing the detection of specific effects of sulphurous acid. Of great importance are especially the rapid oxidation, the quick diminution of sulphurous acid from the substrate by formation of gaseous SO_2 and the formation of addition products with various compounds. The intensity of sulphonate formation depends

on the amount of reactive substances, pH and temperature.

It is generally known that deteriorative changes take place in dried figs, as in other dried fruits, if they are exposed to unfavourable temperature and relative humidity conditions during subsequent storage. These changes are primarily the microbial spoilage and the non enzymic browning (Maillard reaction), along with some other secondary changes (sugaring etc). A survey of literature indicates little information on stability of retailed packages of Calimyrna figs (Baker & Mrak 1938; Barger 1941, Barger *et al.* 1948) many of the works dealing with fresh figs (Claypool & Ozbek 1952; Kim 1981; Pala & Saygi 1990). Barger (1941) had demonstrated that the package in which the fruit was stored had an important influence on moisture adsorption from the storage atmosphere and this in turn affected storage defects such as sugaring and fungi contamination. The concentration of soluble solids in the fruit and the temperature of storage are also important factors affecting the resistance to microbial deterioration (Miller & Tanaka 1963). It has been known for a long time that low temperature is beneficial to dried fruit, particularly after processing. Cold storage has been used to some extent by the dried fruit industry, especially during the summer months (Nichols & Reed 1931).

The inhibitory effect of SO_2 on non-enzymic browning is well known (Stadtman *et al.* 1946c; Stadtman 1948; Schroeter 1966). It has been stated by Ingles (1966) that SO_2, sulphites and bisulphites exert their inhibitory effect by breaking the chain of chemical reactions that leads to the formation of brown pigments. During the storage period dried fruit browned as the SO_2 content decreased. It was stated that the rate of browning is accelerated by increasing temperature and moisture content (Legault *et al.* 1951) and that it is also dependent upon the water activity (a_w) of the product (Labuza & Saltmarch 1978). The activity of sulphites increases with a reduction in pH, presumably because it is the undissociated acid that has the antibrowning germicidal action (Cruess *et al.* 1931; Rahn & Cohn 1944; Rhem 1964; Dyett & Shelley 1966; Banks & Board 1981). The proportion of the free SO_2

in the dissociated form rises from 0.5% at pH 4 to 5.5% at pH 3, so that even quite small shifts of pH towards more acid values greatly enhance the preservative value of added sulphite. Acidity has an important secondary effect: the combination of sulphites with sugars and other compounds is greatly delayed by increasing the acidity - a decrease of only 0.3 pH units roughly halves the rate of combination (Vas & Ingram 1949).

As mentioned previously (Chapter 3), sulphured dried figs of Kymi are marketed in paper containers with a moisture content between 20-25% and a water activity of 0.72-0.80 and are usually kept during marketing at an average temperature of 25°C. Their shelf life is expected to be up to 12 months and this can be determined by the extent of browning, the contamination by souring yeasts and spoilage fungi and the water activity and moisture content.

In order to measure the storage stability of sulphured dried figs under different storage conditions, the objectives of this part of the study were to provide specific information about kinds and rates of physicochemical and microbiological changes that occur during storage under different temperatures and atmospheres and the effect of sulphuring on these changes. The lack of microbial standards for dried figs in Greece, as well as the fact that these products are a good substrate for the growth of microorganisms, specially moulds, led to the undertaking of a study, in which a survey was made of the quantitative and qualitative microbiological aspects of figs of Kymi.

Further studies about the occurrence of toxin-producing fungi in dried figs as well as toxin production and the effect of sulphuring will be discussed in Chapter 5 of this project.

Materials and methods

The materials and methods are given (pp 57-60) in the section B of Chapter 2, Materials and methods.

The method for sulphuring figs used was the exposure to vapours from a 6% w/v SO_2 solution (method IV, p. 79).

Results

Physicochemical analysis

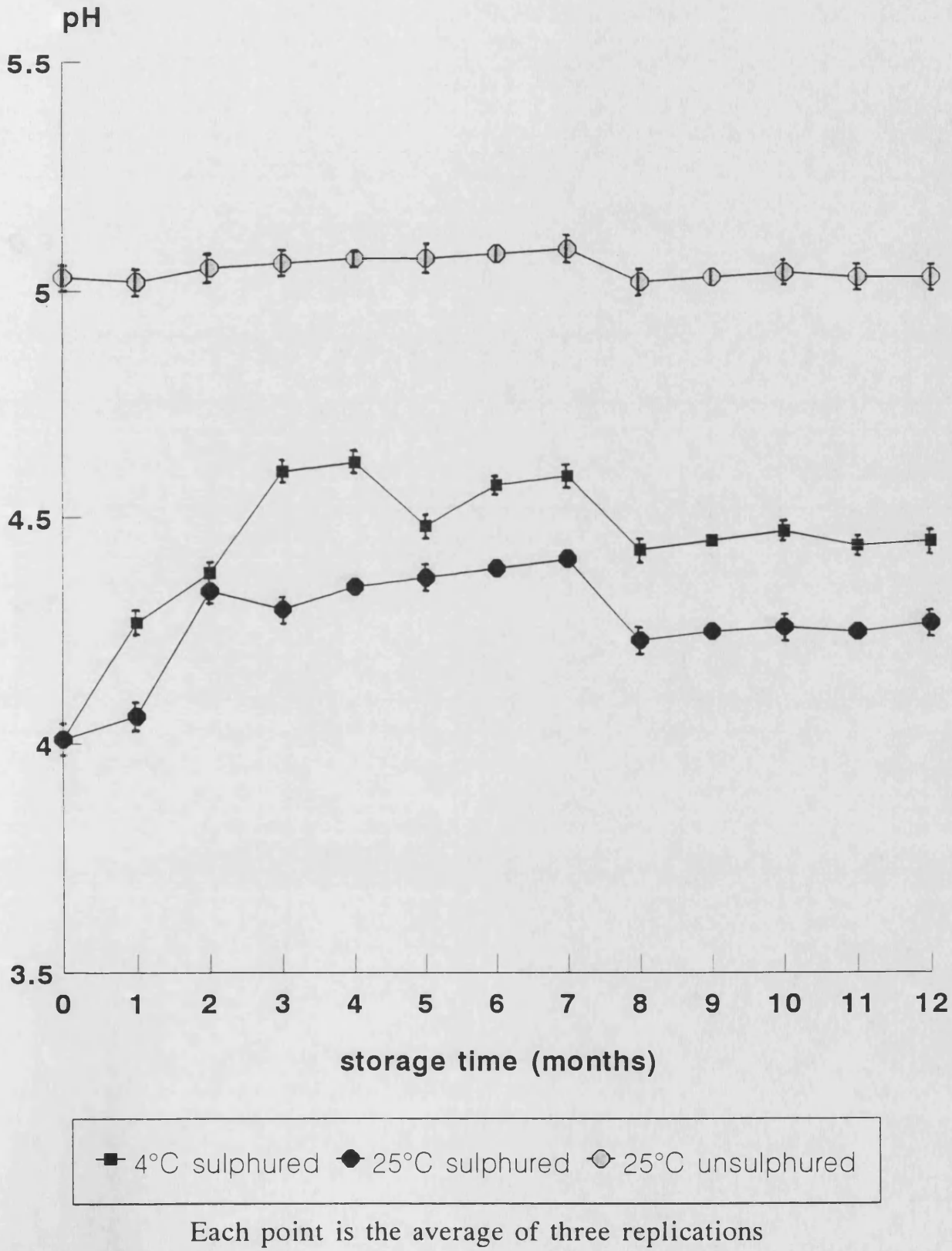
A.Effect of storage temperature and sulphuring

A progressive shift of pH values was observed at the beginning of storage of sulphured dried figs (Fig. 4.1), with cold storage (4°C) resulting to a greater increase in pH (0.6 units after 4 months i.e a 4-fold increase) than warm storage (25°C, 0.4 units at the same time). In both cases however, a decrease was observed after the 7th month of storage (about 0.2 units) followed by a stability of pH values until the 12th month (4.4 and 4.2 final values respectively at 4 and 25°C). During warm storage, the pH values of the figs that have not been sulphured did not change significantly. As expected, the pH is relatively higher than that of sulphured figs, indicating the increased susceptibility of unsulphured figs to deterioration during storage.

The effect of storage temperature on titratable acidity of figs is shown in Fig. 4.2. The acidity is valuable in determining the quantity of organic acids, since the latter do not ionize completely to be measured by pH. Despite the variation of the results and the fact that especially in the beginning of storage pH values increased significantly (see Fig. 4.1), a disanalogous increase in acidity was observed throughout storage, irrespective of storage temperature. One of the possible reasons could be the production of organic acids (acetic, lactic etc) by some microorganisms present in dried figs during storage.

The influence of storage temperature and sulphuring on changes of a_w (water activity) is shown at Fig. 4.3. As it was expected, sulphuring did not affect a_w

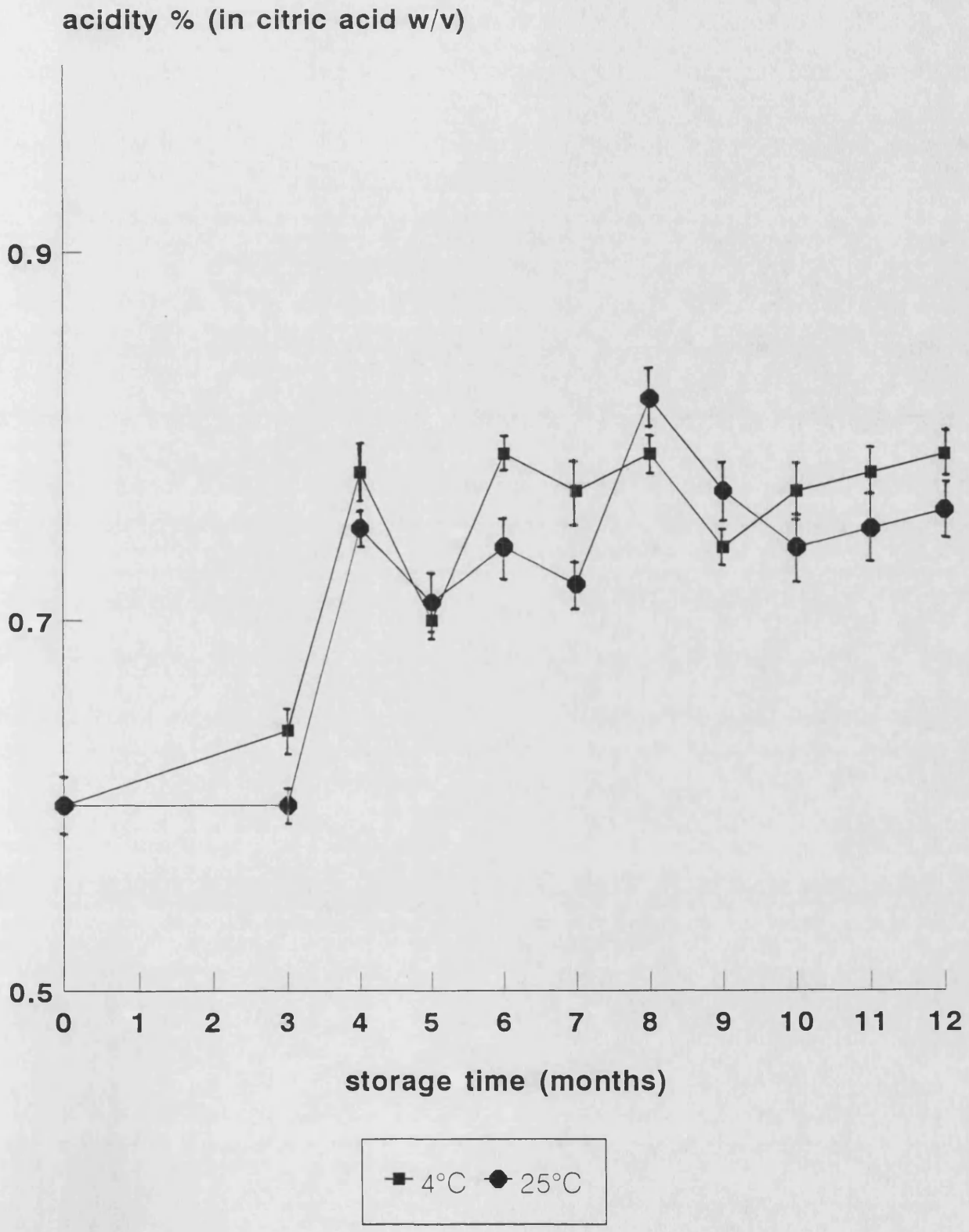
Figure 4.1. Effect of storage temperature and sulphuring on pH of dried figs



Each point is the average of three replications

The statistics were made as described in materials and methods pp.68

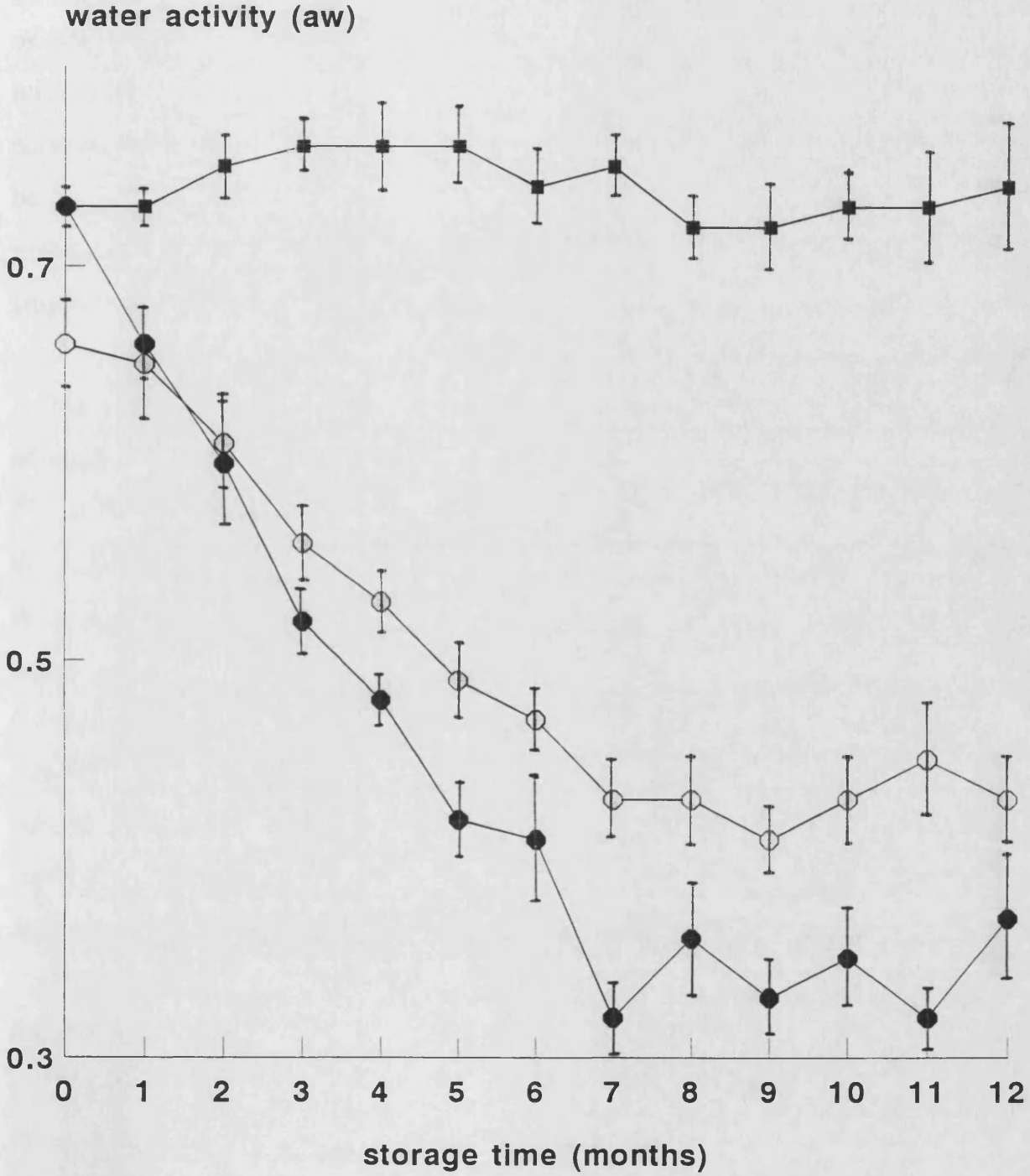
Figure 4.2. Effect of storage temperature on acidity changes of sulphured dried figs



Each point is the average of three determinations

The statistics were made as described in materials and methods pp.68

Figure 4.3. Effect of storage temperature and sulphuring on water activity of dried figs



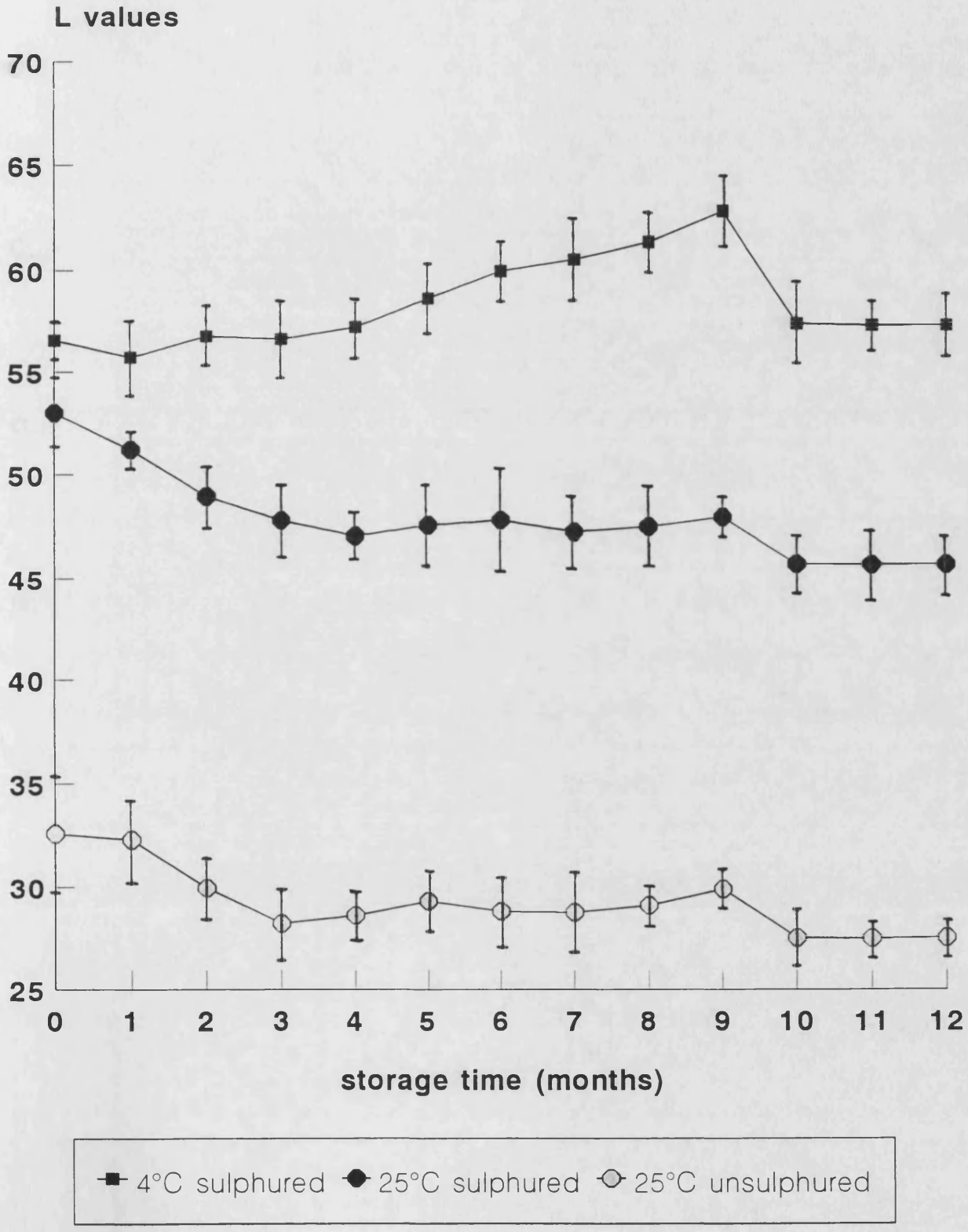
Each point is the average of five determinations

The statistics were made as described in materials and methods pp.68

changes. Figs stored at low temperatures (4°C) had almost a stable a_w through storage at a level around 0.72, where xerophilic moulds and osmophilic yeasts can grow. On the other hand, figs stored at room temperature (25°C), either sulphured or not, had reached an a_w value of 0.5 after only 3 months, which by the microbiological point of view means that from that time on there is no microbial activity, as lower limit for microbial growth is around 0.60 (Bone 1969). It should be pointed out here that drying of the product to such a low level has some undesirable consequences such as "sugaring", that is why cold storage was also studied.

The changes in colour (L=light reflectance, see materials and methods pp. 57) of sulphured dried figs during storage at different temperatures were monitored at Fig. 4.4. It is evident that low storage temperature (4°C) "preserves" the colour of the product, while higher temperatures (25°C) accelerate browning. The observation that the 9 month samples gave a higher reflectance value (i.e lighter) can be attributed to the surface crystalline material occurring during storage ("sugaring") which may possibly give false positive results. The apparent effect of sulphuring is of course the preservation of the yellow colour for a quite long period. Indeed, during the first two months of storage there is a higher rate of darkening more evident at 25°C, possibly because of the simultaneous loss of free SO_2. In the following period, there is a constant inhibition of non enzymic browning either from SO_2 itself (at 25°C) or synergistically from SO_2 and low temperature (at 4°C). The effect of temperature of storage on browning is more evident at Fig. 4.5, where the development of browning at two different temperatures determined by alcohol extractable colour (absorption at 440 nm) according to Nury *et al.* (1960), is presented. The results are in good agreement with the findings of Stadtman *et al.* (1946a,b,c), Barger *et al.* (1948) and Gouda *et al.* (1975). Because of the importance of non enzymic browning on product

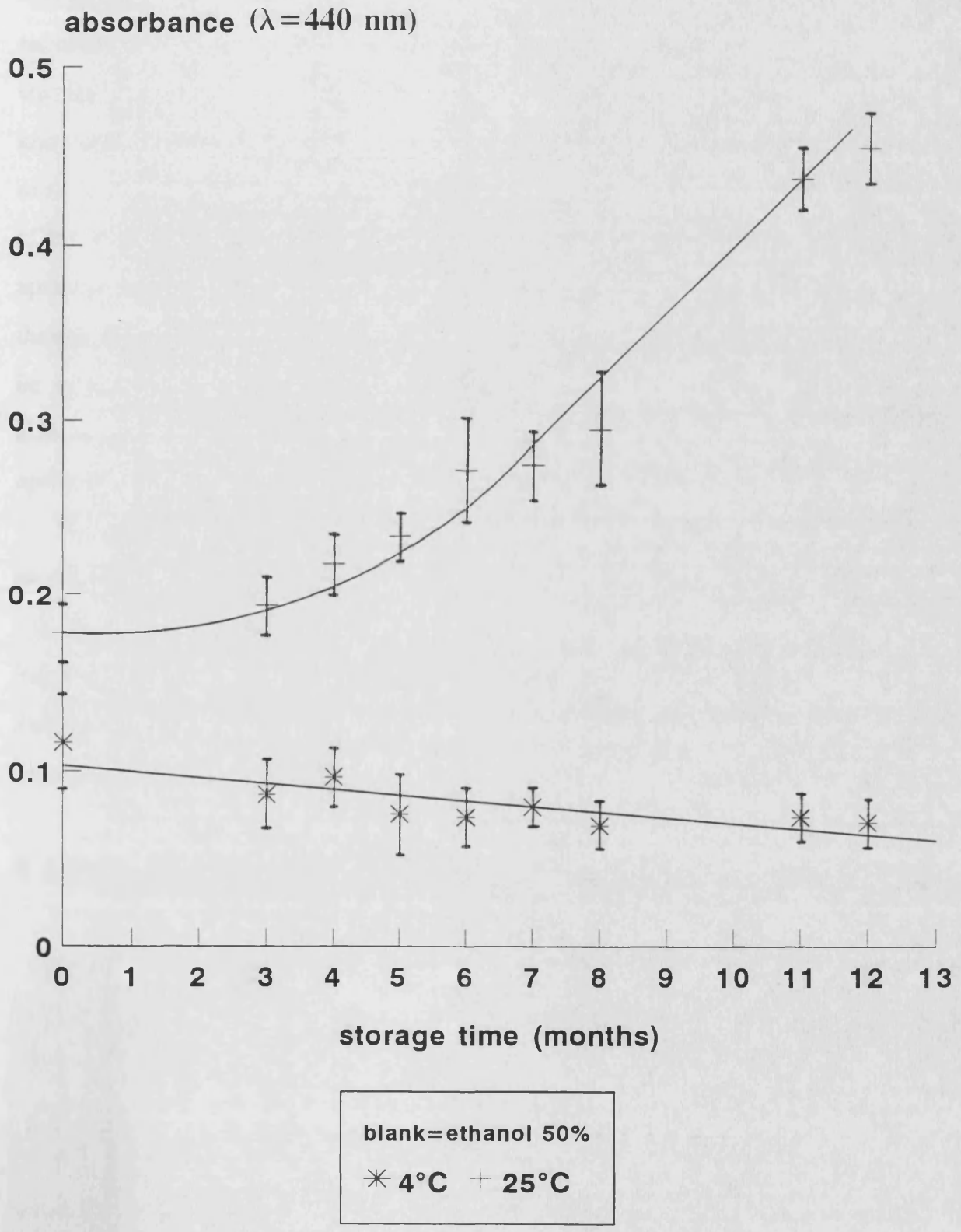
Figure 4.4. Effect of temperature of storage and sulphuring on colour of dried figs



Each point is the average of five replications

The statistics were made as described in materials and methods pp.68

Figure 4.5. Development of browning during storage at 4°C and 25°C of sulphured dried figs



Each point is the average of two determinations

The statistics were made as described in materials and methods pp.68

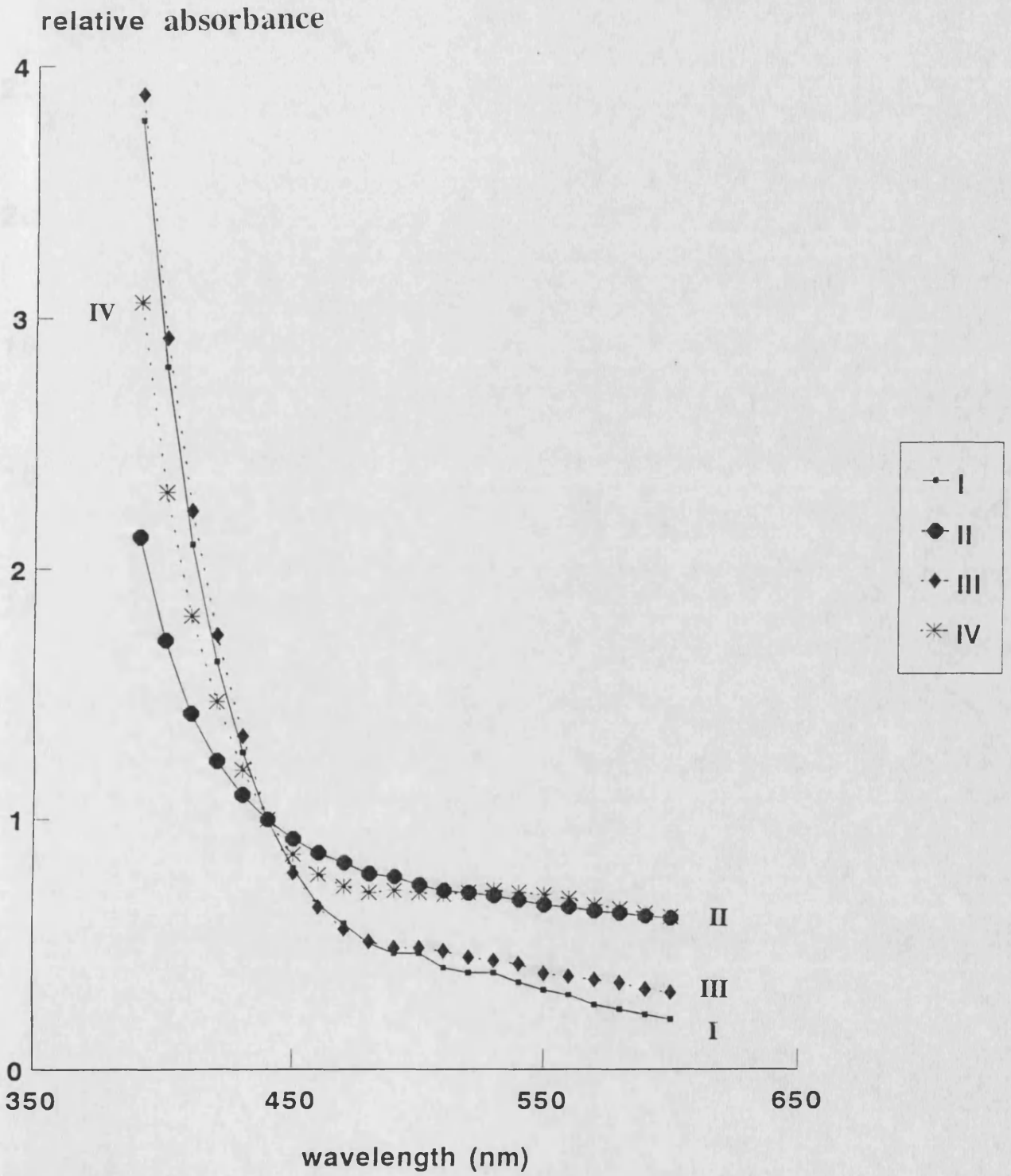
deterioration and quality and the remark that at high temperatures appreciable changes in colour occur during the first 30 days of storage, when 30% of the free SO_2 is lost (see Fig. 3.16), the spectra of figs sulphited and not at 25°C was recorded for that period and the relative absorbance was plotted against wavelength (Fig. 4.6). Light absorption in extracts from figs increases markedly and continuously with decreasing wavelength over the range 460 nm down to 380 nm. Sulphite exerts some effect on the absorption spectra of the extracts. This effect was also observed in pronounced form by Stadtman *et al.* (1946a,c) for dried apricots and Hendel *et al.* (1950) for dehydrated vegetables. It should be noted though that measurements of degree of darkening by photometric procedures would be valid only for fruit with the same initial sulphite content. The above results are also in good agreement with the findings of Nichols *et al.* (1938) obtained for dried apricots.

The packaging material had a consistent effect on retention of humidity of figs as shown in Fig. 4.7. The initial 21.78% level slightly decreased after 6 months of storage, with low density polyethylene having the best retention. The loss was markedly more rapid and great with paper package of the Cooperation. After 4 months, the humidity was diminished almost to the half, resulting in the quick "sugaring" of figs.

B. Effect of the composition of storage atmosphere

The sulphured dried figs were stored at room temperature (25°C) in low density polyethylene bags under 3 different atmospheres: vacuum, 50% CO_2 - 50% air and 100% air and the changes at some physicochemical characteristics affecting their quality was studied. The results are presented in Figures 4.8-4.11. It is clear that the pH of figs stored under CO_2 or vacuum is lower 0.3-0.4 units than that of fruits stored under air (Fig. 4.8). Little, if any were the effects of storage under different

Figure 4.6. Effect of sulphite and browning on absorption spectra for extracts of dried figs



Relative absorbance = absorbance at a given wavelength/ absorbance at 440 nm

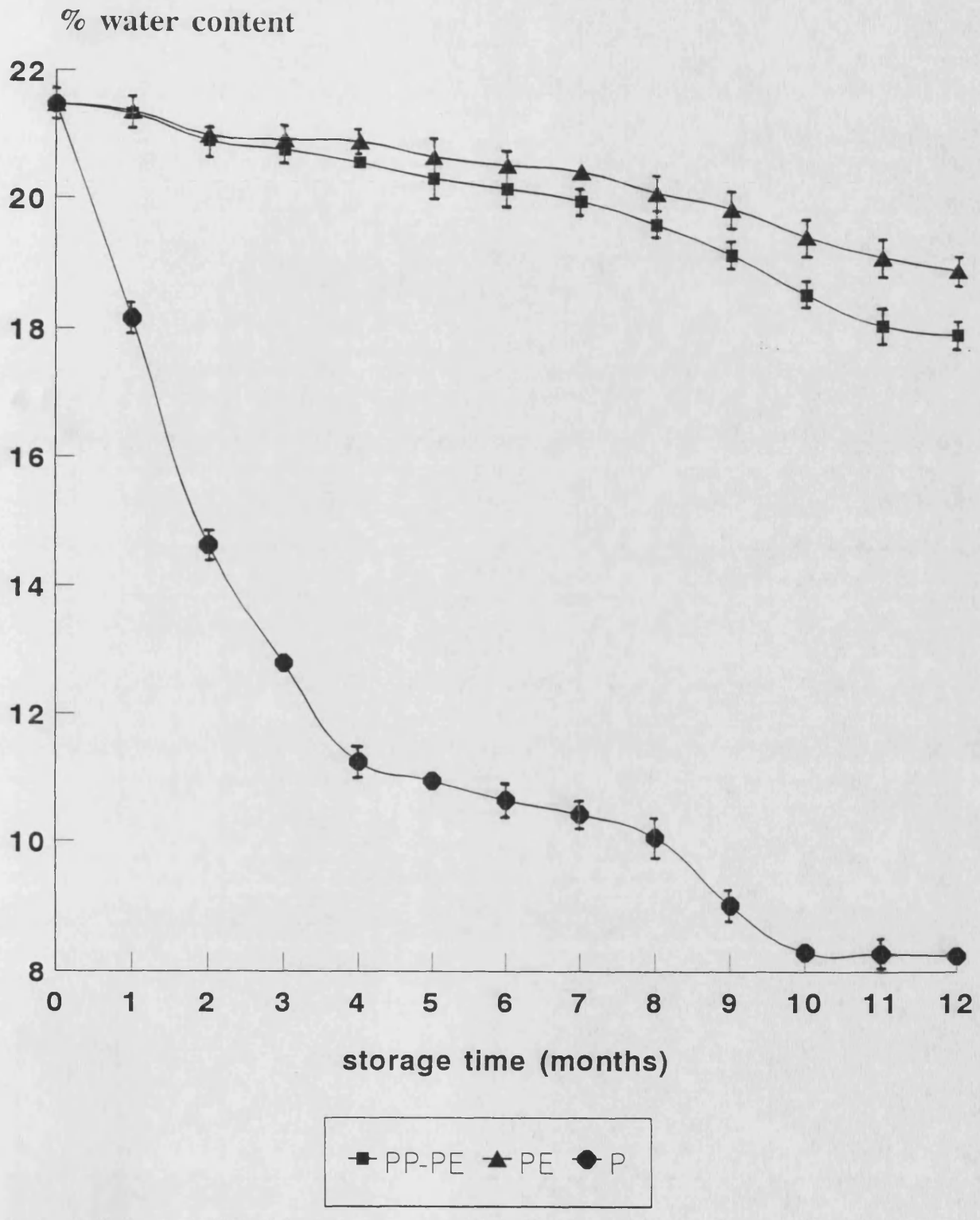
Sulphited figs: **I**. Unbrowned (beginning of storage) with 1400 ppm SO_2 (A_{440}=0.102)

II. After 30 days at 25°C, residual SO_2 content=950 ppm SO_2 (A_{440}=0.079)

Non sulphited figs: **III**. Unbrowned (beginning of storage) (A_{440}=0.122)

IV. After 30 days at 25°C (A_{440}=0.246)

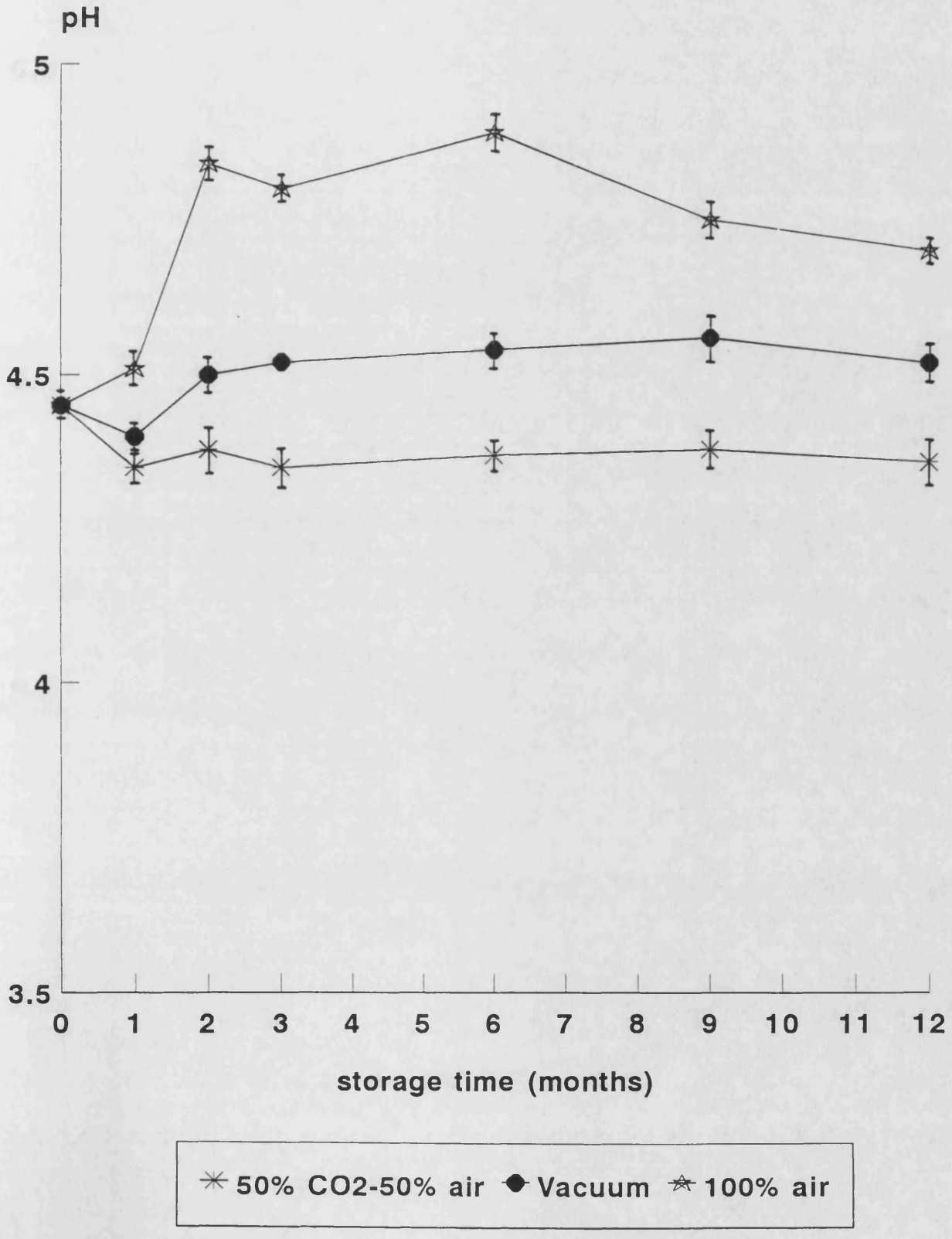
Figure 4.7. Effect of packaging material on retention of water in sulphured dried figs



PP-PE: polypropylene-polyethylene laminate
PE: low density polyethylene P: paper package
Each point is the average of two determinations

The statistics were made as described in materials and methods pp.68

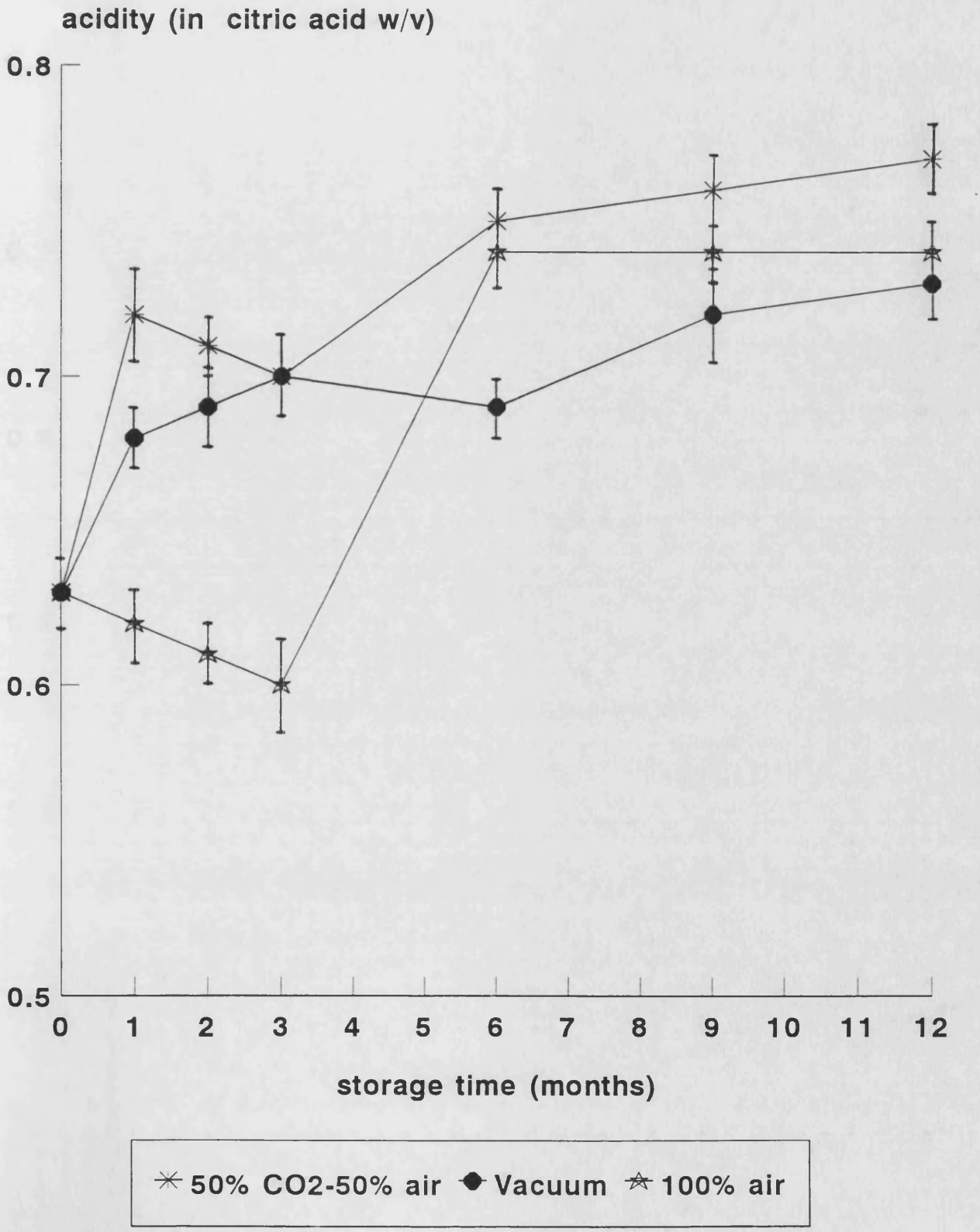
Figure 4.8. Effect of the composition of storage atmosphere on pH changes of sulphured dried figs at 25°C



Each point is the average of three replications

The statistics were made as described in materials and methods pp.68

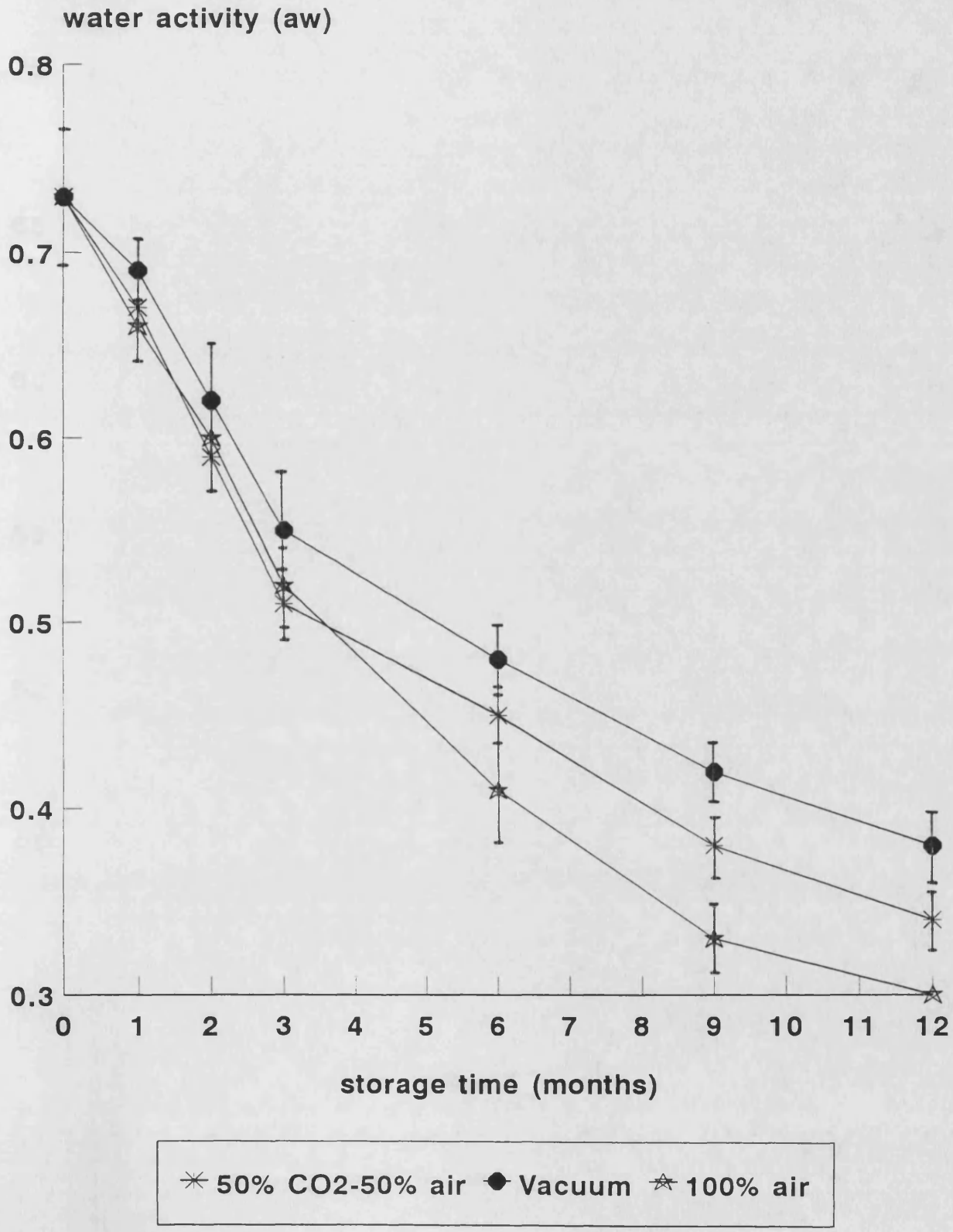
Figure 4.9. Effect of the composition of storage atmosphere on titratable acidity of sulphured dried figs at 25°C



Each point is the average of two replications

The statistics were made as described in materials and methods pp.68

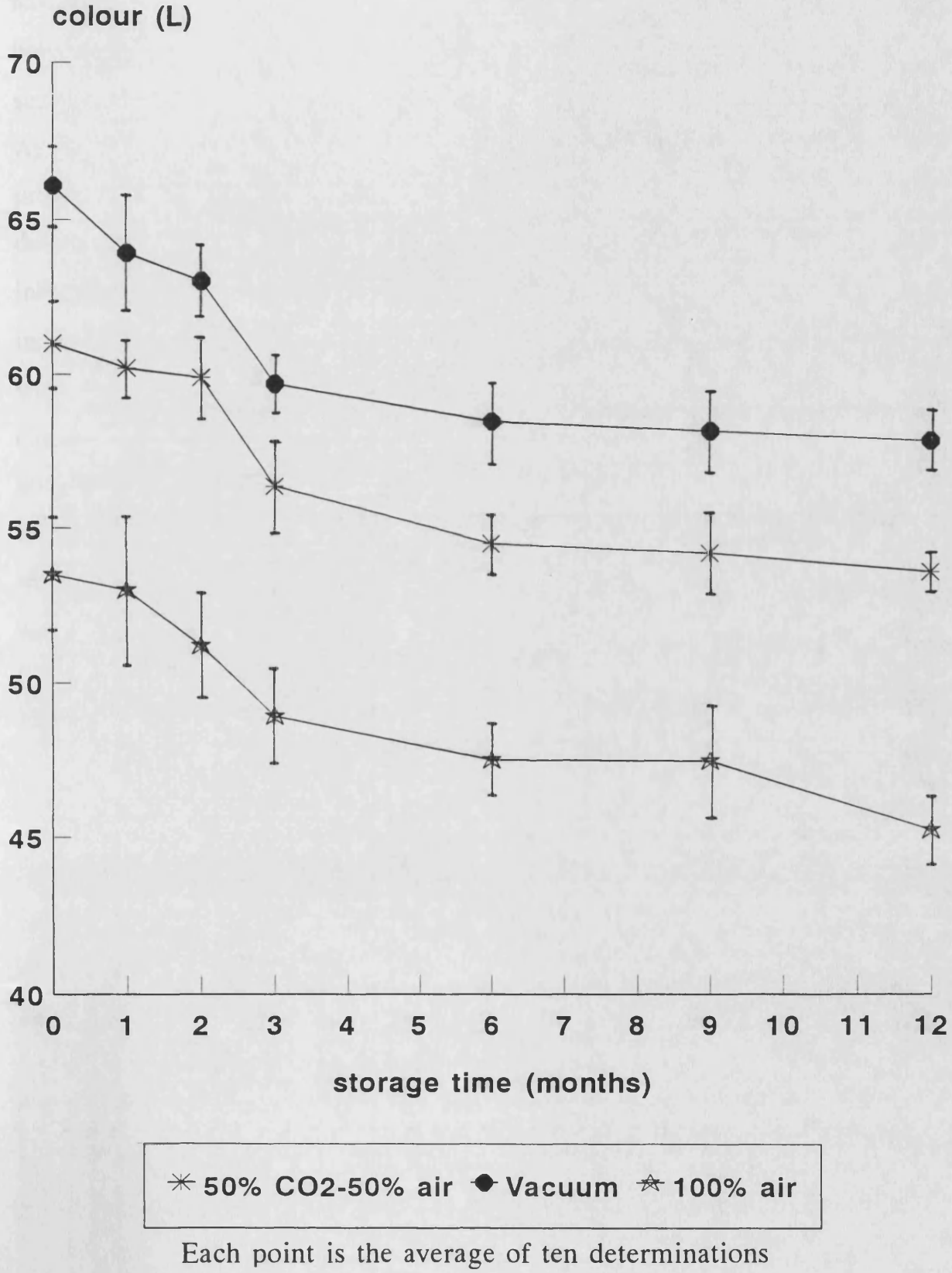
Figure 4.10. Effect of the composition of storage atmosphere on water activity changes of sulphured dried figs at 25°C



Each point is the average of five determinations

The statistics were made as described in materials and methods pp.68

Figure 4.11. Effect of the composition of storage atmosphere on colour changes of sulphured dried figs at 25°C



Each point is the average of ten determinations

The statistics were made as described in materials and methods pp.68

atmospheres to acidity or water activity changes of figs (Figures 4.9 & 4.10). It may seem strange though, comparing Fig. 4.10 with Fig. 4.3 and 4.7 that figs having a low a_w (0.3-0.6) have a quite high water content (>18%). This could possibly be due to the fact that "sugaring" creates a crystalline material on the surface of the product which might affect the water vapour pressure above the fig. As can be seen from Fig. 4.11, the composition of the atmosphere surrounding the product did not play an important role in preventing colour deterioration of the figs during storage. In the case of CO_2, this is primarily due to the fact that its inhibitory effects increase with decreasing temperature as CO_2-solubility is increased at lower temperatures. Unfortunately, it was not possible to find samples with the same initial L value due to dissimilarity of samples, but the rate of colour changes through storage seemed to be the same, irrespective of the storage atmosphere. As it concerns sugars, vacuum storage was found to exert little if any influence on their changes during storage (Table 4.1). On the other hand, an increase in reducing sugar content after the 6th month of storage was observed at samples stored under air or CO_2 with the greatest increase in the samples stored under air. This is probably due to the changes in water content of the samples (see Fig. 4.7 & 4.10).

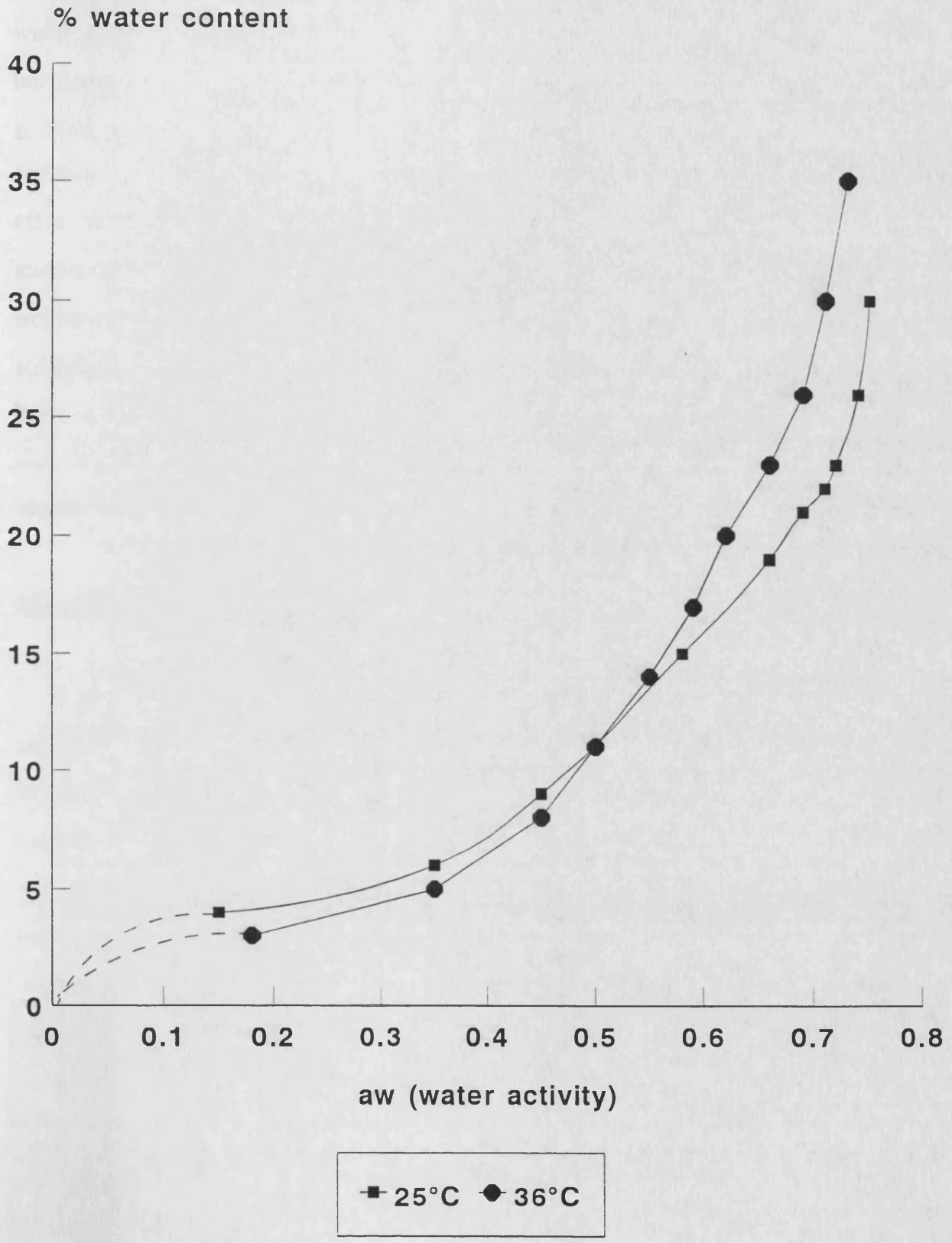
An important factor indicating the quality of a product is the adsorption isotherm, i.e the plotting of the water content of the product against its a_w. The isotherms for dried figs were taken by means of the HUMIDAT thermoconstander at 25 and 36°C (Fig. 4.12), representing temperatures that are commonly used for storage in retail market and in the Cooperation during summer until their sale respectively. The shapes of the isotherms are characteristic of the high sugar foods. The curve has a sigmoid character but as figs like all fruits contain an appreciable amount of soluble solids, they have the highest water contents at high a_w. The slight sigmoid shape of the first part of the isotherms is caused by the water

Table 4.1. Changes in sugar content (reducing-sucrose-total) of sulphured dried figs during storage under different gaseous atmospheres at 25°C.

Time of storage (months)	Atmosphere#	% Sugars (in d.w.b*)		
		Reducing	Sucrose	Total
0	a	68.1	7.1	75.2
	b	68.1	7.1	75.2
	c	68.1	7.1	75.2
1	a	66.6	2.0	68.6
	b	63.5	2.7	66.2
	c	63.7	1.9	65.6
3	a	65.5	1.7	67.2
	b	63.2	1.4	64.6
	c	62.5	1.5	64.0
6	a	73.8	0.9	74.7
	b	70.8	0.5	71.3
	c	74.7	0.5	75.2
9	a	71.7	0.4	72.1
	b	68.7	0.4	69.1
	c	72.1	0.0	72.1
12	a	70.5	0.2	70.7
	b	66.8	0.2	67.0
	c	68.3	0.0	68.3

atmospheres of storage:
a: 50% CO_2 - 50% air
b: vacuum
c: 100% air
* d.w.b.:dry weight basis

Figure 4.12. Sorption isotherm of figs at 25°C and 36°C



sorption of the contained biopolymers (polysaccharides, pectins and proteins) and the sharp increase in water content at high water activities is due to the sugars. At water activities lower than 0.5, the moisture content of figs decreased as the temperature was raised from 25 to 36°C. This is in agreement with the observed general tendency of food isotherms and can be explained by the increased heat of sorption (stronger binding of water) at low water contents. However, the opposite effect was observed at higher a_w and the figs absorbed more water at higher temperature. The change in sorption properties is evidenced by the crossing of the isotherms in Fig. 4.12 at a_w=0.5. This behaviour is attributed to the increased solubility of fruit sugars at higher temperatures. Similar effects of temperature have been observed by Audu *et al.* (1978) and Weisser *et al.* (1982). Our findings are also in good agreement with the findings of Makower & Dehority (1943) for dried vegetables, Bolin (1980) and Saravacos *et al.* (1986) for sultana raisins.

Microbiological analysis

A microbial survey was done for fresh and dried figs in 1991 and 1992. The sampling was done either with cotton swabs in the field (for surface of fresh figs) or with immersion of sample in distilled water in the laboratory. Total viable counts, as well as the enumeration of moulds and acetic acid bacteria were done in this survey by using the appropriate selective/differential media (see Chapter 2 - section b, p.59-60).

During the two years of this survey a total of 92 samples were analyzed (50 of fresh figs and 42 of dried ones) in order to determine the range of some microorganisms occurring on figs. It was found that a few bacteria with moulds were common organisms on fresh figs. The results are shown in Table 4.2. It was found that out of 50 figs, 6 (i.e 12 %) were sterile. In 21 out of 50 samples acetic acid bacteria were found, but at very low counts, making thus disputable the

Table 4.2. The mean value (log cfu/g) of microorganisms of fresh figs, the range (min/max) and the number of samples.

1991

Microorganisms	Number of samples	Mean value	Range
Moulds[1]	23	5.72	4.0-7.61
Acetic acid bacteria[2]	12	0.35	0.0-1.23
Total viable count[3]	23	6.23	4.7-9.60

1992

Microorganisms	Number of samples	Mean value	Range
Moulds[1]	21	6.7	3.1-8.42
Acetic acid bacteria[2]	9	0.13	0.0-0.95
Total viable count[3]	21	7.69	3.7-9.92

1 : Mould counts on Rose-Bengal Chloramphenicol Agar (Oxoid) incubated at 25°C for 3 days
2 : Acetic acid bacteria counts on Acetobacter Agar incubated at 30°C for up to 7 days
3 : Total viable counts on Plate Count Agar (Oxoid) incubated at 25°C for 3 days

accuracy of the values of Table 4.2 concerning those organisms. It should be pointed out though that the isolation and enumeration of acetic acid bacteria in random selected samples without previous enrichment of the substrate is very difficult. It should also be noted that the contribution of the acetic acid bacteria to the endogenous flora was not found as a more extensive microbiological examination of fig should be needed along with isolations and identifications which was not in our objectives of this part of the study. As it concerns some high total viable counts (9.6-9.9), they were found in some soft-ripe and obviously wilted figs which were among the randomly selected samples. The relatively high difference between mould counts (7.6) and total viable counts (9.6) observed in some samples can possibly be attributed to other bacterial counts present in soured figs which can not be isolated and enumerated with the used media. Because of the peculiar structure of the fig (syconium) a study was also done in order to find the changes in microflora at different stages of maturity and between interior and tissue of figs with a closed or open ostiole. The sampling was done with cotton swabs in the field for the tissue and after ethanol sterilization of the tissue for the interior of figs. The results are shown at Figures 4.13 & 4.14. It was found that the more mature the figs are, the more increased microbial population they have. Indeed, the statistical analysis (F-test) revealed that fruits of different maturity differ significantly ($P<0.05$) at the number of microorganisms present. A limited multiplication had been observed between the microflora of the interior and tissue of figs with a closed ostiole, but figs with an open ostiole apart from the increased microbial count also showed a differentiation in the proportions of microorganisms present compared to those with a closed eye (Fig. 4.14). The predominant species are moulds but an appreciable amount of acetic acid bacteria is present as well. This is probably due to the fact that a microflora is introduced through the ostiole by fig wasps and insects (*Carpophilus, Drosophila* etc.) which persists and develops throughout the period of ripening. The relatively high population of the

Figure 4.13. Influence of maturity of figs on their microflora

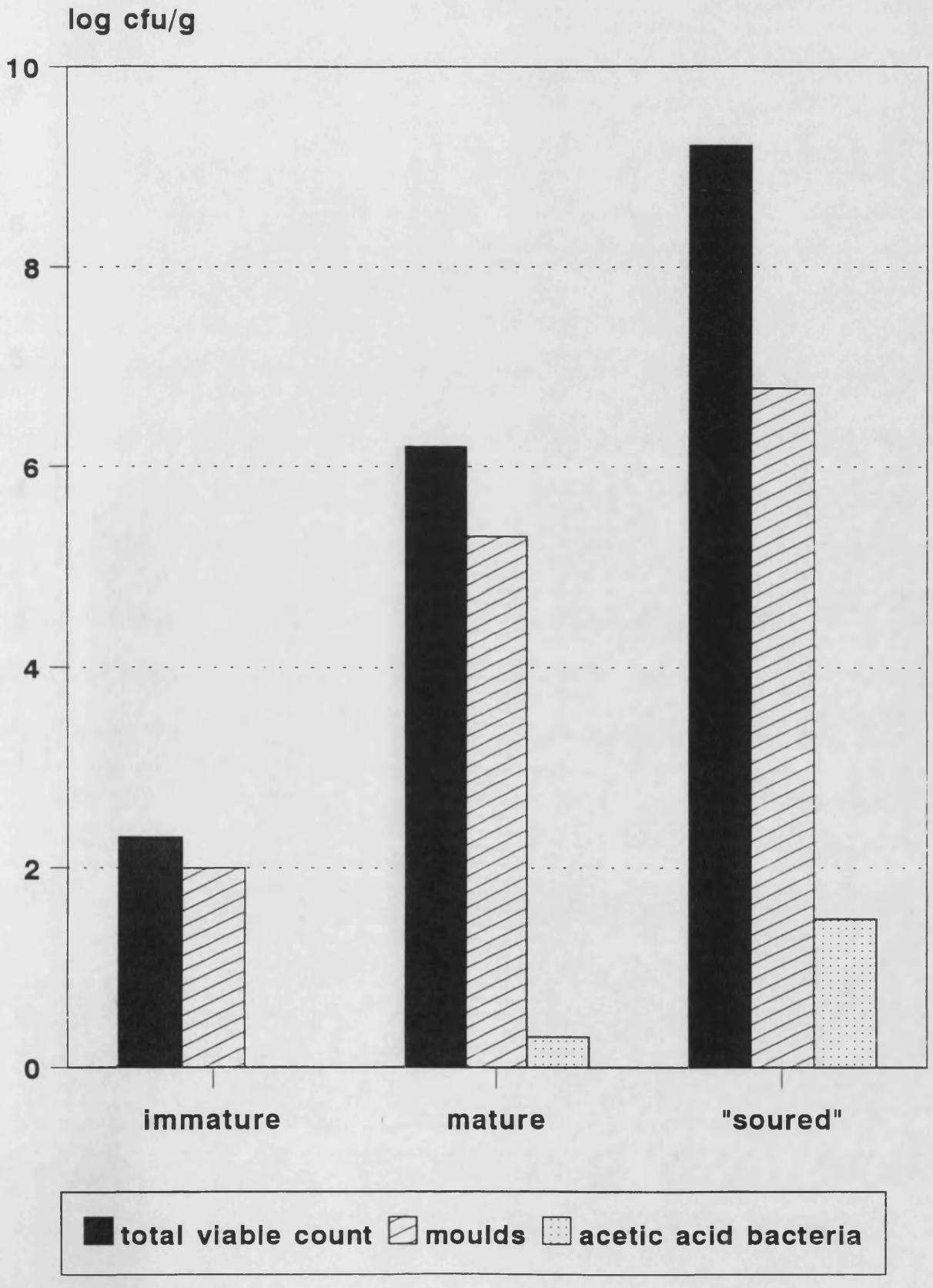
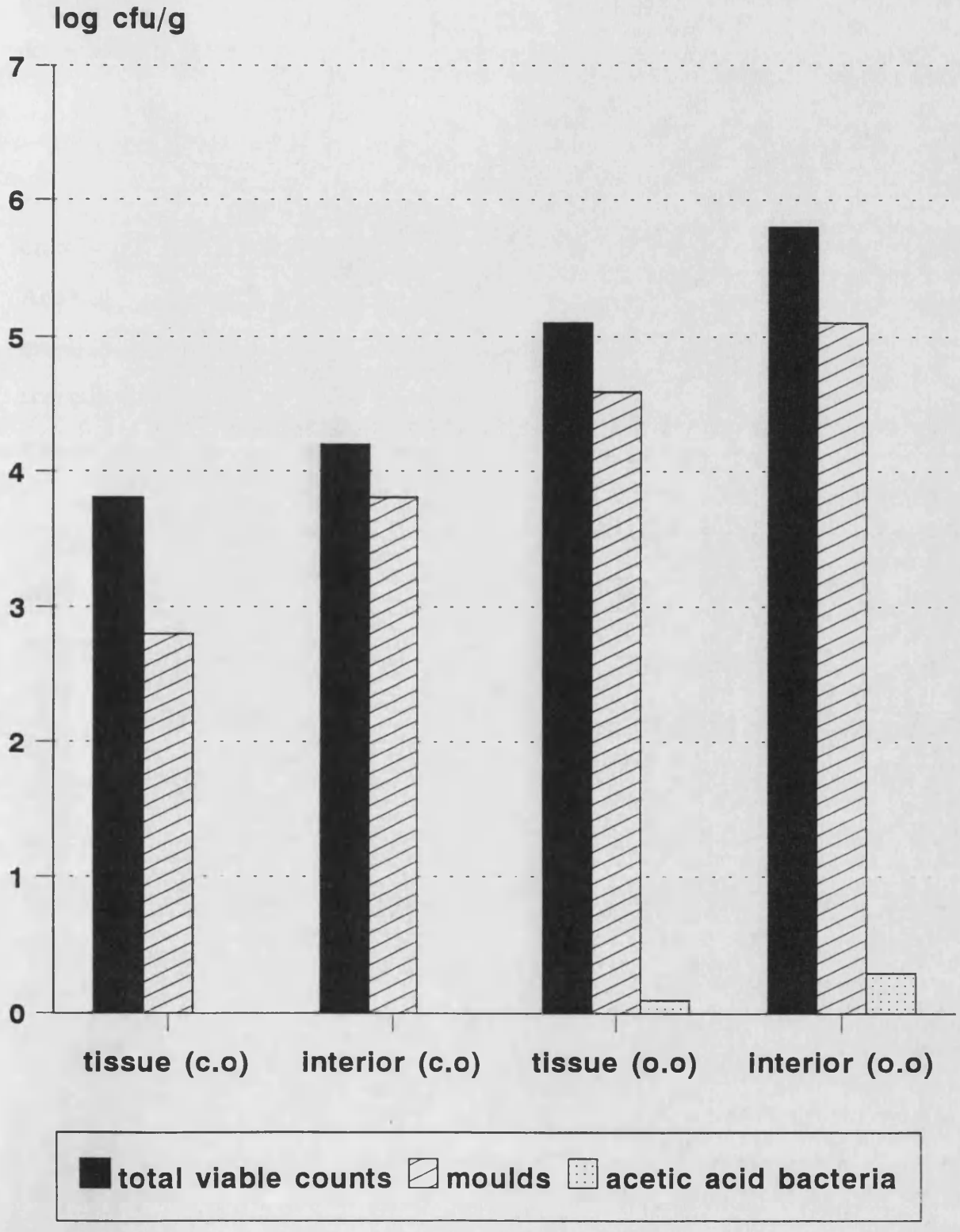


Figure 4.14. Changes on microflora between interior-tissue of fresh figs with an open or closed ostiole



c.o : figs with a closed ostiole
o.o : figs with an open ostiole

interior of figs with sealed eyes is probably attributed to the fact that closing of the eye, might be expected to create a more humid condition in the fig, favouring germination of mould spores. These results agree with the data published by Phaff & Miller (1961) and Miller & Phaff (1962) for Calimyrna figs.

As it concerns dried figs, generally they have a reduced microbial load compared to the fresh ones (Table 4.3). Their microbial population differs from that of fresh ones in the occurrence of xerophilic moulds and the absence of acetic acid bacteria. Anyway, the microbial counts of the sulphured fruits were found by all means much lower than the ones of the non sulphured fruits. The above results are in accordance with the findings of King *et al.* (1968) and Wehner & Rabie (1970) who also observed low microbial counts on dried fruits.

The effect of temperature of storage on microbial counts of dried figs is shown in Fig. 4.15. A low temperature for storage of sulphured dried figs inhibits microbial growth, something which is visible from the beginning till the 4th week of storage. On the other hand, storage at room temperature (25°C) increases the total viable population of the product. It can be seen that there is a growth of number of moulds at RBC medium during the first 4 weeks of storage of sulphured dried figs at 25°C, when water content and a_w are still enough high (a_w=0.6) for growth of xerophilic moulds and osmophilic yeasts. During the second month of storage the microbial count is dramatically reduced maybe because of the higher rate of a_w reduction (see Fig. 4.3).

The effect of sulphuring on the microbial count of dried figs stored at 25°C is presented at Fig. 4.16. Except a limited reduction at the microbial count of sulphured dried figs compared to those that had not been sulphured due to the antimicrobial action of SO_2, the shapes of the curves are similar, i.e a growth

Table 4.3. The mean value (log cfu/g) of microorganisms of dried figs, sulphured or not, the range (min/max) and the number of samples.

Sulphured dried figs

Microorganisms	Number of samples	Mean value	Range
Moulds[1]	19	3.37	2.1-5.3
Xerophilic fungi[2]	16	28[&]	10-42
Acetic acid bacteria[3]	-	n.d[#]	n.d
Total viable count[4]	20	4.02	2.4-6.92

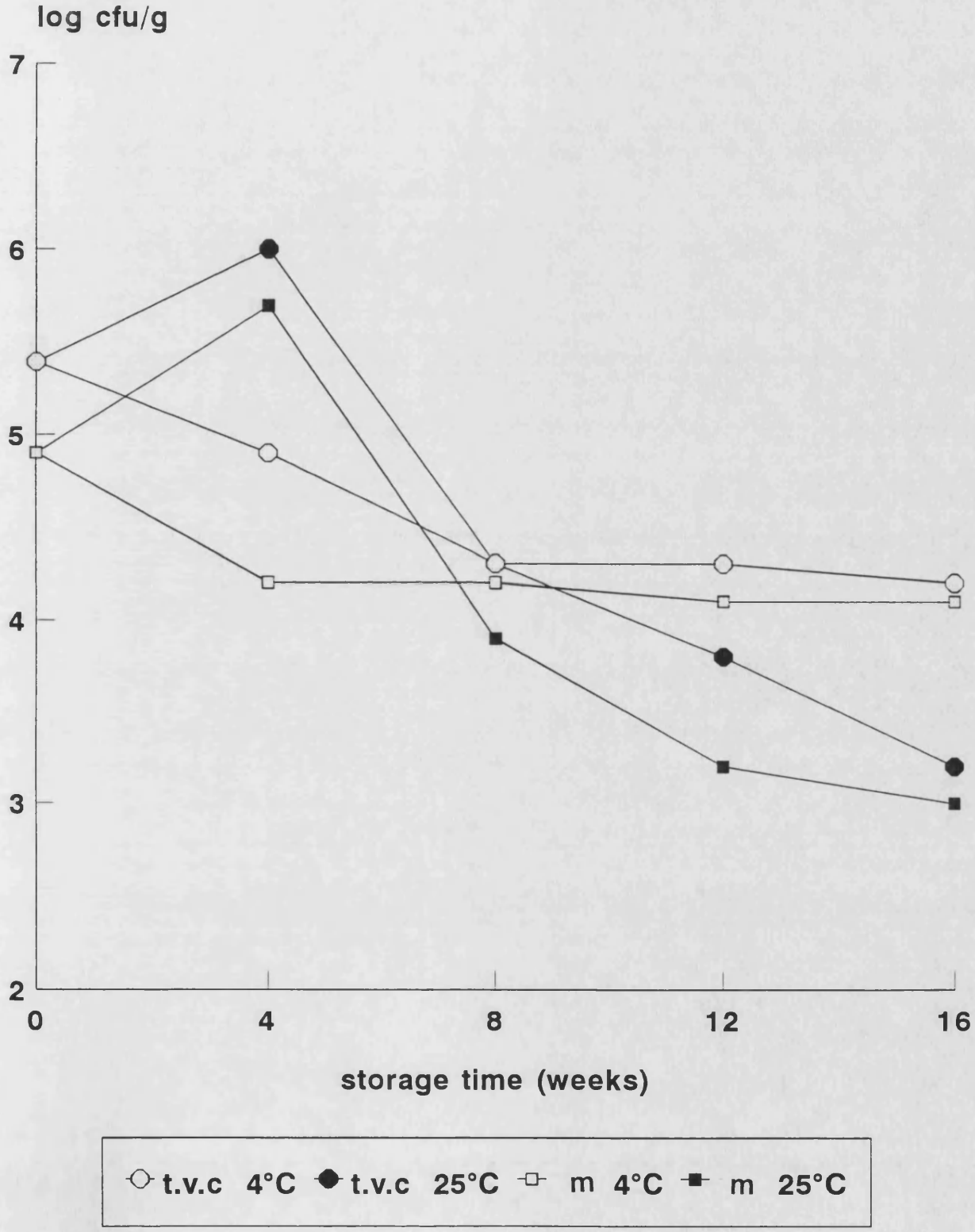
Unsulphured dried figs

Microorganisms	Number of samples	Mean value	Range
Moulds[1]	21	5.3	3.0-6.92
Xerophilic fungi[2]	18	69[&]	7-126
Acetic acid bacteria[3]	-	n.d	n.d
Total viable count[4]	22	5.97	2.2-7.35

1 : Mould counts on Rose-Bengal Chloramphenicol Agar (Oxoid) incubated at 25°C for 3 days
2 : Xerophilic fungi counts on Dichloran-Glycerol 18 Agar (Oxoid) incubated at 25°C for up to 6 days
3 : Acetic acid bacteria counts on Acetobacter Agar incubated at 30°C for up to 7 days
4 : Total viable counts on Plate Count Agar (Oxoid) incubated at 25°C for 3 days

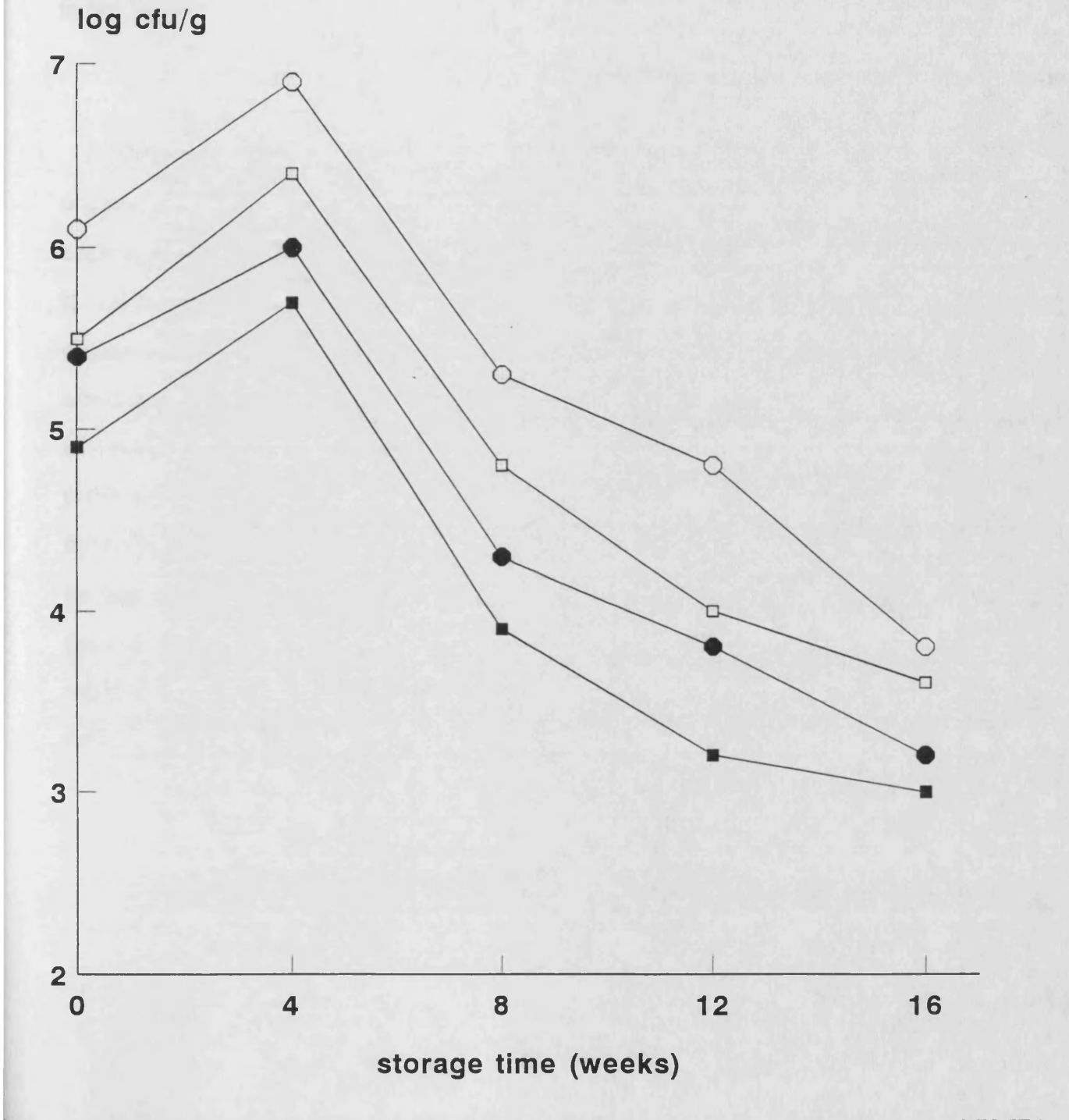
& : number of xerophilic colonies per g of food
# : not determined

Figure 4.15.Effect of temperature of storage on microflora of sulphured dried figs



t.v.c : total viable counts on PCA
m : mould counts on RBC

Figure 4.16. Effect of sulphuring on microflora changes during storage at 25 °C



t.v.c :total viable counts on PCA
m : mould counts on RBC

during the first month of storage and then a drastic reduction. It was noteworthy that during the first 30 days, even though the free SO_2 level was enough high, it failed to inhibit the growth of moulds which are more resistant to sulphur dioxide.

In this project I have provided some basic knowledge concerning the groups of organisms existing on sulphured dried figs. Through the period of the survey I understood that maturity and condition of figs could influence their microbial flora. It was important to note that the sulphuring procedure, the use of insecticides, the meteorological conditions before harvesting and during sun drying could also affect the groups of organisms. It should be also kept in mind that the temperature of storage affects the growth of microorganisms in dried figs. As mentioned previously, our aim in this part of the study was to provide a basic concept for the microbiology of dried sulphured figs that is why no further differentiation of groups was made even though it is known that other microorganisms might be present (such as lactic acid bacteria). It should also be pointed out that our main interest in microbiology of figs was the occurrence of *Aspergillus flavus* group of moulds which can produce the aflatoxins.

Discussion

According to Stadtman *et al.* (1946a,b,c) the edible storage life of dried fruit can be defined as the time required for the fruit to darken to such an extent that it is no longer generally acceptable. Among the factors which affect the rate of deterioration and consequently the storage life are temperature, moisture, pH, SO_2 content, available oxygen and the previous history of the fruit (Barger 1941; Legault *et al.* 1951; Claypool & Ozbek 1952; Morris *et al.* 1979). Thus it is important to study the effect of those factors to shelf life of dried figs. It was evident by the results of this part of my study that the browning of dried sulphited figs stored at 25°C usually proceeds through an apparent induction period followed by an approximately linear path (Fig. 4.5). It is noteworthy though that at 25°C the darkening of figs, indicated by an absorbance greater than 0.3, occurred when 60-65% of their original total SO_2 content is lost, that is after 6-8 months (see Fig. 3.15 of Chapter 3).

Stadtman *et al.* (1946a,b) first suggested that the quality of the food product must be taken into account in selecting a storage temperature. While it would seem desirable to store all fruits at refrigerator temperatures (4°C), this is not always best for the maintenance of desirable quality in some foods. In every case, the success of storage temperature depends to a great extent upon the R.H of the storage environment and the presence or absence of gases such as carbon dioxide and oxygen. Our studies revealed that "sugaring" appeared to be the major disadvantage for cold storage of packaged dried figs. Otherwise, cold storage helped retain the yellow colour and to a lesser extent the original moisture content of the figs. The results from this part of my study also confirmed that the combination of sulphite and low moisture greatly prolongs the time that dried figs can be held without detectable change at an ambient storage temperature. It is important though that even a_w of the fruit is set at low values (0.60), the fruit

should be stored under conditions of R.H that do not allow the food to pick up moisture from the air and thereby increase its own surface and subsurface a_w to a point where microbial growth can occur. In addition to inhibiting the growth of microorganisms, lowering the a_w may also influence the rates of enzymic and chemical changes in foods (Schwimmer 1980; Eichner 1986).

In the processing and marketing of dried fruits only a restricted moisture content range is used. For figs the moisture range is 17-25%, therefore only the a_w range of 0.55 -0.75 is of commercial interest (Fig. 4.12).

It is known that with regard to a_w, the maximal browning reaction rates in fruits occur in the 0.65 to 0.75 range (Troller & Christian 1978, Labuza & Saltmarch 1978, Rockland & Nishi 1980). However, some other authors (Eichner & Karel 1972; Warmbier *et al.* 1976) suggested that the maximum rate for Maillard browning occurs in the 0.4 to 0.5 a_w range. This was evident in our studies also (Fig. 4.5). The maximum rate of browning occurred after the third month of storage when a_w level is below 0.5 (Fig. 4.3).

Our results, along with that of Stadtman *et al.* (1946a,b,c) support the evidence that although SO_2 retards, it does not prevent deterioration. In some cases (sugar-amino acid interaction) it is an inhibitor, whilst in other cases (ascorbic acid - amino acid interaction) it is merely a retarder of brown pigment production. Our studies also support the evidence that temperature and time were more important factors than the composition of atmosphere surrounding the dried fruit in colour deterioration and shelf life of product (Figures 4.4 , 4.11).

Although sorption isotherm provides important information about shelf life it can not be used as a measure of the storage stability, as the estimation of water is quite difficult and important errors can occur if standard methods are not strictly observed. However, sorption isotherm is quite helpful in understanding the effect of a_w on non enzymatic browning. At low a_w, water is tightly bound to surface polar sites by chemisorption and is generally unavailable for reaction and solution

(Labuza & Saltmarch 1978). Also at low a_w the physical state of the sugars may have an important effect on the sorption properties. Amorphous sugars are known to sorb more water than the crystalline materials (Chinachoti & Steinberg 1984). Due to complex food composition, theoretical prediction of the isotherms is not possible and experimental measurements are necessary. Our studies revealed that the maximum stability for storing dried figs at 25°C might be expected when ambient moisture is maintained between 20-32% (Fig. 4.12). As shape of sorption isotherm is strongly dependent on sugar content of fruit, further studies are needed to examine the effects of maturity and processing of fresh figs to the a_w and subsequently the isotherm.

The role of reducing sugars to the storage stability of a dried product is well documented (Eheart & Mason 1967; Hodge 1953; Gehman & Osman 1954; Kim 1981). They can be a good source of energy for foodborne microorganisms. The higher the reducing sugar content the greater the rate of browning during storage (Copley & Van Arsdel 1964). The lower the reducing sugar level the greater the increase in shelf life. Since glucose has the highest binding-SO_2 power, the binding percentage is increased as reducing sugar content increases. Also, the increase in fructose and simultaneous lowering of glucose gives a substrate with an energy source capable of supporting the growth of most microorganisms but with a reduced binding capacity towards sulphur (IV) oxospecies (Carr *et al.* 1976) .

It is known that the populations of viable moulds on sound dried fruits are relative low, one of the reasons being that many fruits are treated with SO_2 prior to drying to prevent browning and the levels that are commonly absorbed (1000-4000 ppm) destroy a very high percentage of the contaminating organisms (King *et al.* 1968). Another reason is that drying is a lethal process for many microorganisms, especially when the organisms are being exposed simultaneously to ultraviolet rays from the sun (Shorey *et al.* 1989). The fact that many factors could influence the

endogenous flora of figs makes almost impossible to study all of them together in a sort period of time. In my study, the statistical analysis (one way analysis of variance) revealed that the physiological stage of the fruits influenced the counts of moulds examined in this survey. Counts made on non-sulphited samples were much higher than those made on sulphited samples, (Table 4.3, Figure 4.16) reflecting the effectiveness of sulphites to reduce the numbers of microorganisms. However, it must be emphasized that the survey during second year had been conducted during a very dry season, when the chances for active microbial growth on the products were quite low.

Another factor that should be considered in the study of microflora of figs is that when the fruit is lightly pollinated, the characteristic microflora does not always develop. Miller & Phaff (1962) found that 82% of figs of the Adriatic variety, which do not require pollination, had sterile internal tissue.

It is of interest though that the natural acidity of fruits, which is below these required by many spoilage organisms, may be thought of as nature's way of protecting plant from destruction by microorganisms.

The influence of SO_2 levels on deteriorative changes of figs is profound when the results in this phase of the study are compared to those of Chapter 3 for SO_2 loss. Thus, it can be concluded that vacuum packaging does not provide the complete answer to retail dried fig packaging and under suitable conditions (initial SO_2 between 1400-2000 ppm, a_w not exceeding 0.75, package under air in plastic bags), it is possible to produce sulphured dried figs with a shelf life up to 12 months, the length of storage being a function of storage temperature.

CHAPTER 5
Aflatoxins in Greek dried figs

Introduction

Aflatoxins, the mycotoxin group most commonly found in agricultural food commodities, have firstly been detected in dried figs in the mid-1970s (Anon. 1974). Buchanan *et al.* (1975) found that aflatoxigenic moulds can grow vigorously on ripe fig fruits. At appropriate temperature and moisture content, spores of *Aspergillus flavus* (which are abundant in the environment) can germinate and resultant hyphae can infect the inner tissues of fig fruits. Rapid fungal colonization and aflatoxin accumulation presumably continued until fungal growth was stopped by lack of moisture in the dried fruit. Further, fig fruits accumulated on a dry-weight basis aflatoxin levels comparable to those found in peanuts, soya beans, corn, wheat, rice and cotton seed (Diener & Davis 1969). Following these findings, Morton *et al.* (1979) reported that dried figs, as well as apricots, raisins and pineapples, were potential substrates for the occurrence of aflatoxins, with figs being the best substrate for growth of mould and aflatoxin formation. Presence of high concentrations of aflatoxin in figs imported into Switzerland (Anonymous 1986) led the Swiss Federal Office for Public Health, in 1988, to set the legal limit for aflatoxin in figs at the same level as for nuts, ie 1 ng/g of aflatoxin B_1 and 5 ng/g for the sum of toxins B_1, B_2, G_1 and G_2 (Akerstrand & Moller 1989). Askin and Kösker (1980) at their studies for growth and toxin production of *Aspergillus flavus* in Turkish dried figs have been observed that aflatoxin was produced on the second day of incubation and reached a maximum on the fourth and sixth day decreasing then gradually. Reichert *et al.* (1988) and Steiner *et al.* (1988) found a positive correlation between the occurrence of aflatoxins in dried figs and a bright greenish yellow fluorescence under ultraviolet light. Gilbert (1989) reported that mould infection of Turkish figs probably occurs when the fruit

fall to the ground, and suggested that ensuring that the moisture content is brought as rapidly as possible below the critical water activity for fungal growth was the only reasonable measure that can be taken in practical terms for prevention. Another report from workers in Turkey (Boyacioglu & Gönul 1990) apparently indicated that aflatoxin contamination of dried figs was highly correlated with the poorer grade of the fruit. Sharman *et al.* (1991) reported a high incidence and high levels of contamination of dried figs and fig pastes from Turkey with aflatoxins B_1, B_2 and G_1.

The problem that aflatoxin could pose in commercial fruit production depends on the frequency of infection with *A. flavus* as well as the aflatoxin levels accumulated in fruits. Incidence of infection is presumably influenced by factors as : (i) location and abundance of spore inoculum (ii) efficiency of spore distribution (iii) ability of spores to penetrate fruits (iv) pathogenicity of the fungus in host fruits and (v) suitability of the host fruit as a substrate for aflatoxin production and accumulation. As described on literature review (pp 33-44) the production of aflatoxins will vary with the substrate, the strain of *Aspergillus flavus* used and storage conditions (temperature, moisture, surrounding atmosphere, pH, a_w). As also mentioned on literature review (pp 16-28) and Chapter 3, bisulphite is a highly reactive chemical and an acceptable food additive. The ability of bisulphite to degrade aflatoxins B_1 and G_1 was first recognised by Doyle & Marth (1978a,b). They observed that potassium bisulphite solutions buffered at pH 5.5 degraded pure aflatoxins B_1 and G_1. The reaction between aflatoxin B_1 and sodium bisulphite yielded almost quantitatively (91 %) a light yellow, highly fluorescent water soluble product with similar fluorescence to that of aflatoxin B_1 and B_2 but less toxic than aflatoxin B_1. They concluded that treating foods with 2000 ppm of bisulphite may degrade moderate amounts of aflatoxin B_1 and aflatoxin G_1. They also observed that the reaction rate of aflatoxin G_1 is faster than that of aflatoxin B_1. Besides bisulphites concentrations, there is a marked increase in aflatoxin degradation with increasing

temperature. These observations were expanded by Moerck *et al.* (1979) and Hagler *et al.* (1982) who found destruction of aflatoxin with low and high levels of bisulphites respectively.

The mechanism of bisulphite degradation of aflatoxin B_1 is unknown, but a single major product was isolated and its tentative identification as a sulphonate (aflatoxin B_1S) formed by addition across the double bond in the furofuran ring system of aflatoxin B_1 was reported (Hagler *et al.* 1983; Yagen *et al.* 1989). Aflatoxins B_2 and G_2 were found not susceptible to the action of bisulphite, again supporting the suggestion that bisulphite acts at the unsaturation in the furofuran ring system of aflatoxin B_1.

As previously described (Chapter 3) figs of Kymi were first sulphured, then sun dried and finally stored in warehouses until sold. High relative humidity and temperature during summer time enhances the growth of microorganisms so that dried figs, stored improperly could provide a suitable medium for the growth of toxigenic strains of *Aspergillus flavus* and the production of aflatoxins.

Our aim in this part of the study was to investigate: 1) whether *Aspergillus flavus* is pathogenic to fig fruits var. Kymi and if so, at what stages are they susceptible 2) whether fig fruits are a good substrate for aflatoxin production and accumulation and 3) the effect of drying, sulphuring and storage on aflatoxin accumulation. This work was pioneering in the sense that the Greek literature does not include any reference to similar studies.

Materials and methods

Some of the materials and methods are given at the section c (pp. 61-68) of Chapter 2.

1) *In vitro* growth of *Aspergillus flavus* and production of aflatoxins

The fruits that were tested were fresh figs, sulphured dried figs and non-sulphured dried figs, in order to examine if there is any effect of sulphuring on the growth of the fungi.

5 samples of each fruit type were placed in separate autoclaved 250 ml flasks and sealed with sterile cotton plugs. The samples were previously cut with a sterile knife into small pieces. 25 g sample and 75 ml distilled sterile water were placed aseptically into the flasks. 4 samples of each type were autoclaved for 20 min at 121°C. The flasks were then cooled to room temperature and inoculated with the aflatoxigenic strains (*A. parasiticus* ATCC 26862). One control sample for each type was also prepared. These were not inoculated but were otherwise carried through the experiment.

Flasks were inoculated with 1 ml of a highly concentrated spore suspension prepared by adding sterile 0.01% Tween 80 to a 4-day PDA plate culture of *A. flavus* and detaching spores by gentle scraping with a sterile loop. Uniform inoculation was thus insured by providing an excess of inoculum, with all flasks inoculated from a single source and by even distribution of the spores through vigorous shaking of each flask after the inoculum had been introduced. After inoculation all samples were kept in the dark incubator for 30 days at 25±2°C (optimum for *A. flavus* growth and aflatoxin production) and observed daily for growth of mould. The presence of *A. flavus* group, in the sense of the series consisting of *A. flavus* Link and *A. parasiticus* Speare was verified after culture on Czapek Dox Agar and AFPA mediums and subsequent stereoscopical examination based on colony colour and gross morphology of conidial heads (Raper & Fennell 1965). In control samples (not inoculated) showing natural contamination, the production of aflatoxin was detected using the aflatoxin production medium (APA) (see Chapter 2, pp 61). At the end of 30-day period, 50 ml $CHCl_3$ was added to each sample to attenuate the mould and to extract any aflatoxin present. The samples were mechanically shaken for 20 min and the solution was filtered through a Whatman paper. 25 ml of each chloroform filtrate was used for further analysis

for total aflatoxins by using the TD 100 total aflatoxin test kit of Biocode (York, UK) as described in Section c of the Chapter 2 (pp. 62-65) omitting the sample preparation part.

2) Susceptibility of fresh figs

In order to determine the susceptibility of fig fruits to invasion by *A. flavus*, figs at four stages of maturity were taken as samples:

a) green fruits, immature but ready to eat

b) firm-ripe fruits that had about fresh market maturity

c) soft-ripe fruits, completely softened but without visible wilting and

d) shrivelled-ripe fruits, ready to fall from the tree and obviously wilted.

The samples were inoculated with *A. flavus* stock cultures. Two inoculation methods were used: (i) injection of a spore suspension into the fruit cavity (syconium) with a syringe (ii) dusting a dry conidia-talc mixture onto the surface of fruits with a hair brush. Control samples were not inoculated in order to examine the possibility and the stage at which natural contamination of fresh figs occurs. Ten replicates of each fruit type were done. After inoculations, the fruits were placed in a constant temperature incubator (30±3 °C, temperature existing during summer time). Every day the figs were examined macroscopically for mould growth. Once a visible growth was detected, verification and identification of the mould at AFPA and Czapek Dox agar mediums was done. Whole fig samples were drawn at 15, 30, 45 day intervals and examined for aflatoxin production by using the TD 100 total aflatoxin test kit of Biocode, as described in section c of Chapter 2 (pp 62-65).

3) Association of aflatoxin contamination with blue green yellow fluorescence

A long-wave ultra violet lamp (wavelength 360 nm) was used. Figs were randomly selected from pallets (total number of figs inspected 225, equivalent to 5.4 kg). The samples giving blue green yellow fluorescence were further analyzed for total aflatoxins with TD 100 total aflatoxin test kit of Biocode.

4) Effect of temperature and composition of storage atmosphere on growth of *A. flavus* group and production of aflatoxins

In order to better assess the influence of sulphuring, drying and storage conditions on growth of *A. flavus* and occurrence of aflatoxins in fig fruits, sulphured and non sulphured dried figs kept in plastic bags from polyethylene (plastic A) were stored at two different temperatures (4 and 25°C) under 100% air and at three different atmospheres (vacuum, 100% air and 50% CO_2-50% air) at 25°C for 12 months. The samples (five for each case) were inoculated with fresh stock cultures of *A. flavus* when they are fresh and soft ripe, by an injection of a spore suspension into the fruit cavity. Control samples were not inoculated. The fruits were then sulphured and dried as usual and stored under the above described conditions. Every three months a package was drawn and examined for mould growth at AFPA and Czapek Dox Agar mediums and for aflatoxin production with the TD 100 total aflatoxin test kit of Biocode.

5) HPLC analysis of dried figs for aflatoxins

Analysis was carried out using a Hewlett Packard Series II 1090 HPLC chromatograph connected to a HP 1046A programmable fluorescence detector. The column was a Lichrosphere RP 18, 5μm (25 cm x 4 mm i.d) of MZ analytical, protected by a precolumn, thermostated at 35°C and operated at 0.8 ml/min flow rate, with a mobile phase water/acetonitrile/methanol (58:30:12 by volume). The solvents were HPLC grade. The detector was operated at excitation and emission wavelengths of 364 and 434 mm respectively. Quantification was based on peak area.

The chromatographic profile of extracts from fresh figs, dried sulphured figs and dried non sulphured figs were checked by HPLC. Reference aflatoxin standards diluted in toluene-acetonitrile (98:2 by volume) were analyzed with the same conditions (described above) by HPLC as standards or were added to a sample extract (spiked sample). The extraction of aflatoxins was made as described at pp. 65 of Chapter 2. All samples were derivatized with trifluoroacetic acid before HPLC analysis and injected the same day.

Results

The samples taken were tried to be representative of a lot. The presence of visible *A. flavus* is a good indicator of the possibility of detectable aflatoxins in the sample.

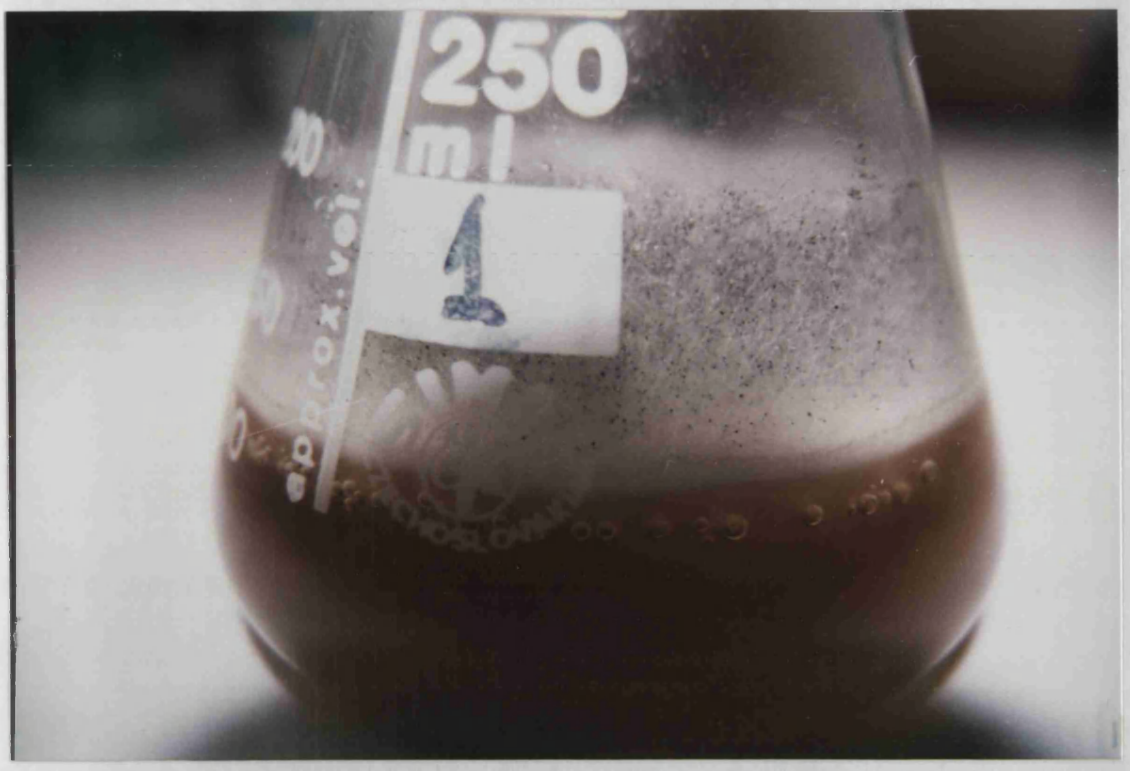
Growth of *A. flavus* and production of aflatoxins

From the three kind of samples (pulp from fresh figs, dried sulphured and not sulphured figs inoculated and autoclaved as described in materials and methods pp. 141) tested for *in vitro* growth of *A. flavus,* the mold was detected at 2 out of 4 fresh samples (Fig 5.1) and at one out of 4 dried not sulphured figs (Fig. 5.2) but at different time intervals (after 66 and 72 hours for the fresh samples 1 and 4 respectively and after 20 days for the dried non sulphured sample 7). The fresh figs showing infection from *A. flavus* were more mature than the ones showing no mould growth, supporting thus the findings of Buchanan *et al.* (1975) that mold was not growing on unripe fig fruits. In none of the 3 control samples (not inoculated) mould growth was detected. The subsequent analysis for aflatoxin production showed only one fresh sample to have total aflatoxin produced in levels in total of 5ppb (ng/g). The other samples were either producing aflatoxins in lower than 1 ppb levels or are completely free from aflatoxins. The results for inoculated samples are presented in Table 5.1. The pH of the fresh samples was 4.45-4.58 and of dried sulphured 3.98-4.10.

As it concerns the susceptibility of fresh fruits to *A. flavus* infection, the results show that all soft-ripe and shrivelled ripe figs supported the growth of mould at this temperature and quite humid conditions (a_w of samples 0.98-0.99). In 70% of the firm ripe figs (7 out of 10) a mold growth was detected, whilst only one out of 10 (10%) green fruits showed visible mold growth but much more later than the ripe ones. That is an evidence that mold growth is favoured when the fruit is ripe enough, as Buchanan *et al.* (1975) had also found. Regarding the production of

Fig.5.1. *In vitro* growth of *A. flavus* in fig fruits: (a) fresh figs after 5 days incubation (b) fresh figs after 7 days incubation at 25°C.

(a)



(b)

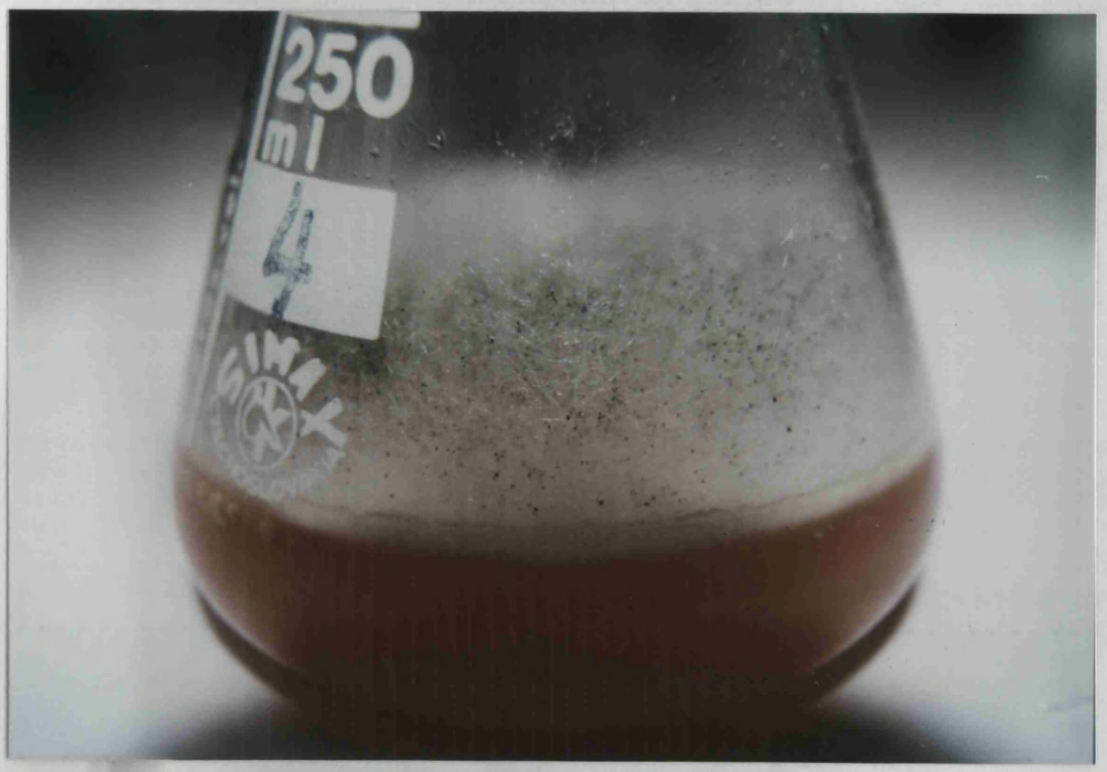


Fig.5.2. *In vitro* growth of *A. flavus* in fig fruits: dried non sulphured figs after 20 days of incubation at 25°C.

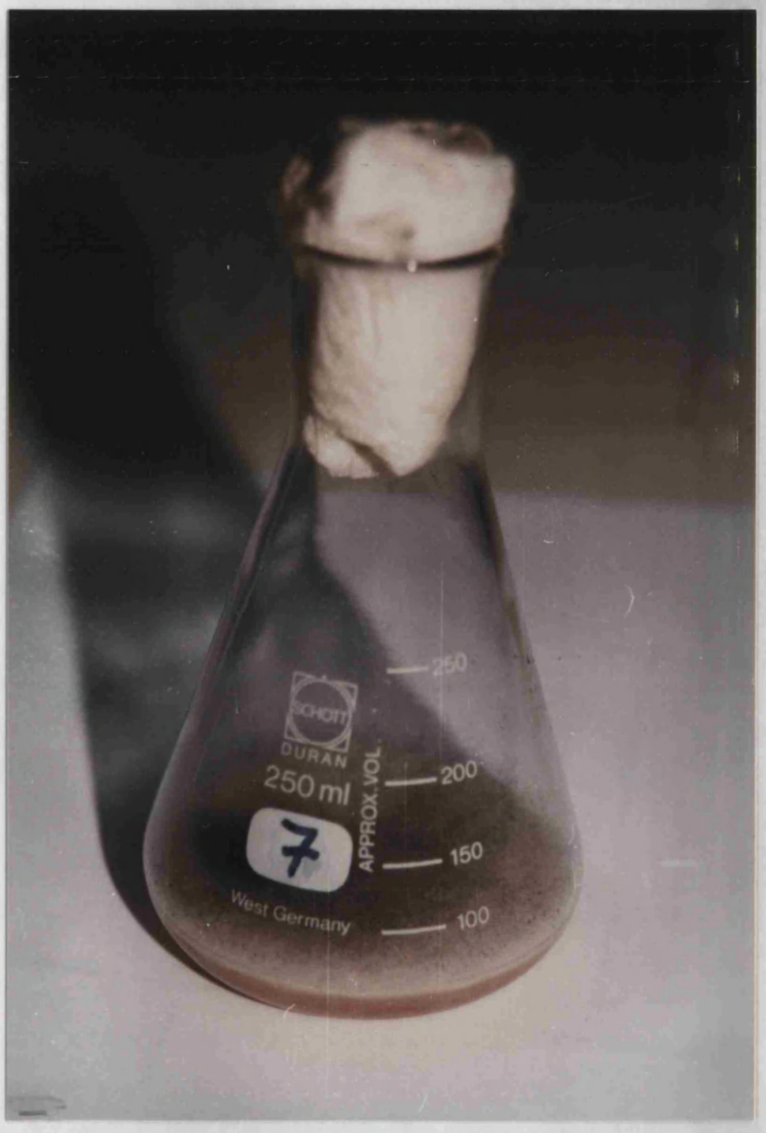


Table 5.1. *In vitro* growth of *A. flavus* and production of aflatoxins in figs.

Sample	Number of inoculated samples[#]	*A.flavus* detected (number of samples)	High total aflatoxin present in ng/g (ppb)[*]
fresh	4	2	5(1)[+]
sulphured dried	4	0	-
non sulphured dried	4	1	-

[#]: the samples were inoculated with *A.parasiticus* ATCC 26862 as described in materials and methods pp.141

[*]: detection limit of the method 1 ppb

[+]: number of samples in parenthesis where aflatoxin was determined in levels > 1ppb

aflatoxins, all the *A. flavus* inoculated figs showed an aflatoxin accumulation and differ not such in the quantity of aflatoxin accumulated but in the time of presence of this quantity. That is probably due to the fact that fruits begin with a different stage of maturity and the more mature fruits produce aflatoxin more quickly than the others which need first some time to mature. It was interesting though that some control samples showed a mold growth when ripe (Fig. 5.3a). This was identified as an *A. flavus* group in the sense of the series consisting of *A. flavus* Link and *A. parasiticus* Speare (Raper & Fennell 1965), giving the reverse yellow colouration in AFPA medium (Fig. 5.3b). The fact that 35-85% of the strains are aflatoxigenic (Wei & Jong 1986) makes the subsequent cultivation in APA medium indespensable. The mold was cultured on Czapek Dox Agar and then tested for its ability to produce aflatoxin in APA medium, but none of the isolated from control fruits *Aspergillus* showed production of aflatoxin. It was interesting that mould infection occurred in spaces near the syconium (figs with open ostiole) or where a visible wilting (injury) on the fruit was, indicating that these are the susceptible parts for mould infection.

Aflatoxin contamination of figs is associated with a blue-green-yellow fluorescence under UV light (Steiner *et al.* 1988). The results presented at Table 5.2 reveal that 2 % (2 out of 100) of the sulphured and 4 % (5 out of 125) of the non sulphured dried figs showed BGY fluorescence at the beginning of storage. Of these, none of the sulphured figs and only 3 non sulphured figs gave positive results when further analysed for aflatoxins suggesting that the fluorescence is not necessarily an indication of aflatoxin production, as Steiner *et al.* (1988) suggested. Of course the screening still remains a rapid method applicable to fig producers for discarding the suspected figs. As storage time passes, the percentage of figs showing BGY fluorescence remains the same for the sulphured ones, but increases slightly through time (from 4 to 5.6 % after 6 months) for the non sulphured figs.

The effect of sulphuring, drying and storage conditions on *A. flavus* growth and aflatoxin production is presented in Tables 5.3-5.5. The influence of sulphur

Fig.5.3. Susceptibility of fresh figs to *A.flavus/parasiticus* infection: (a) control sample showing a mold growth (b) characteristic reverse yellow coloration of *A.flavus/parasiticus* in AFPA medium.

(a)

(b)

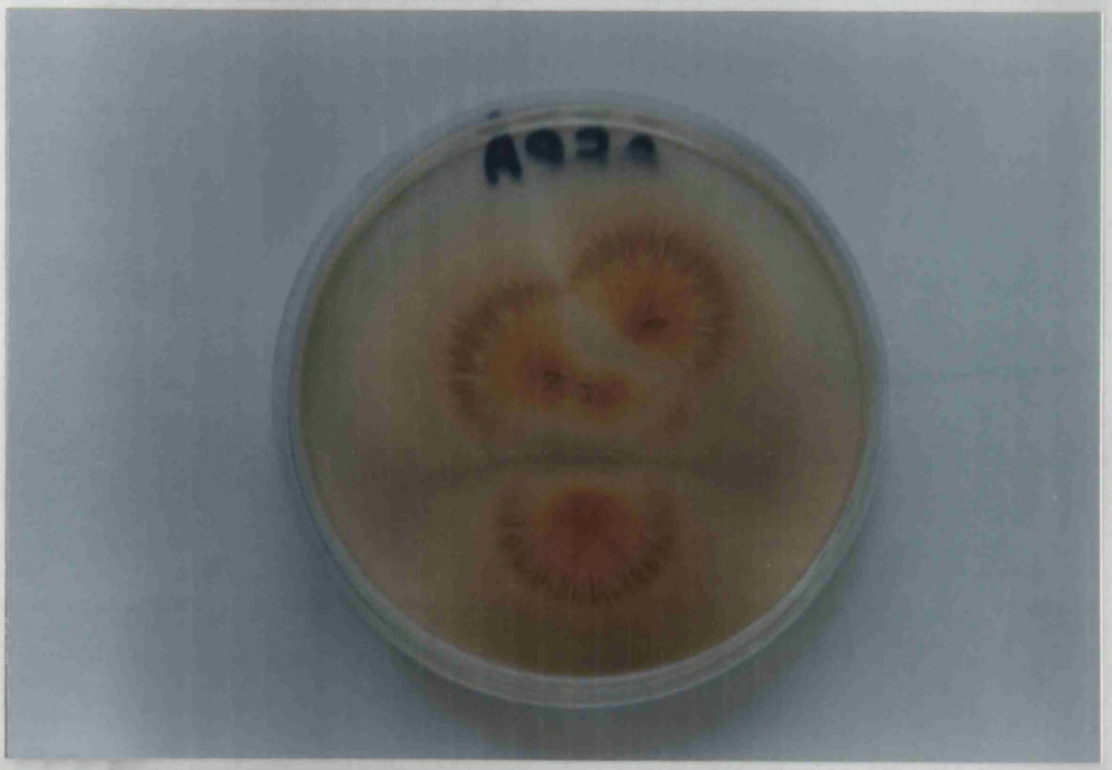


Table 5.2. Association of BGY fluorescence with aflatoxin production.

Type of sample	Number of samples showing BGY fluorescence	Number of samples showing aflatoxin production
dried sulphured* (100)		
at zero time	2	-
2 months after	2	-
4 months after	2	-
6 months after	2	-
dried non sulphured* (125)		
at zero time	5	3
2 months after	5	3
4 months after	6	3
6 months after	7	3

*: Number of samples tested in parenthesis

Table 5.3. Effect of sulphuring on *A.flavus* growth and aflatoxin production during storage of dried figs at 25°C and under air.

Time (months)	Treatment*	Growth of *A.flavus*	High Total Aflatoxin production (ng/g)
0	a	-#	N.D&
	b	+	10
3	a	-	N.D
	b	-	10
6	a	-	N.D
	b	-	10
9	a	-	N.D
	b	-	10
12	a	-	N.D
	b	-	10

*treatments: (a) figs sulphured by the traditional method (avg 1400 ppm SO_2 at the beginning of storage)
(b) non sulphured figs

- : no growth
+ : moderate growth (1-100 colonies per g of fruit)

& N.D.: not detected, or present in levels < 1 ng/g.

Table 5.4. Effect of temperature on *A.flavus* growth and aflatoxin production during storage of dried non sulphured figs under air.

Time (months)	Temperature (°C)	Growth of *A.flavus*	High total Aflatoxin production (in ng/g)
0	4	+#	20
	25	+	20
1	4	-	10
	25	++	50
3	4	-	10
	25	-	50
6	4	-	10
	25	-	50
9	4	-	10
	25	-	50
12	4	-	10
	25	-	50

- : no growth
\+ : moderate growth (1-100 colonies per g of fruit)
++ : extensive growth (> 100 colonies per g of fruit)

Table 5.5. Effect of the composition of atmosphere on *A.flavus* growth and aflatoxin production during storage of dried non sulphured figs at 25°C.

Time (months)	Atmosphere*	Growth of *A.flavus*	High total Aflatoxin production (in ng/g)
0	a	+#	20
	b	+	20
	c	+	20
1	a	++	50
	b	-	20
	c	-	20
3	a	-	50
	b	-	20
	c	-	20
6	a	-	50
	b	-	20
	c	-	20
9	a	-	50
	b	-	20
	c	-	20
12	a	-	50
	b	-	20
	c	-	20

* composition of atmosphere: (a) 100% air
(b) 50% carbon dioxide - 50% air
(c) vacuum

- : no growth
+ : moderate growth (1-100 colonies per g of fruit)
++: extensive growth (> 100 colonies per g of fruit)

dioxide on growth of *A. flavus* and aflatoxin production is evident from the results given in Table 5.3. None of the sulphured samples examined showed a mold growth during the 12-month period, even at the beginning of storage, when the temperature is quite favorable (25°C), but a_w is near the lower limits for aflatoxin production (a_w of samples 0.77-0.78). In the absence of SO_2, a moderate growth of mold was observed at the beginning of storage, followed by a relatively low aflatoxin production (higher amount 10 ppb for total aflatoxins). No growth of mold was detected during the subsequent storage period and that could be explained by the fact that a_w requirements for mold growth are much higher than the one of samples during the storage period (according to Fig. 4.3. of Chapter 4). Hence, the stability of aflatoxin level is expected, since the storage conditions do not support growth of mold and further production of aflatoxins. The above results are in accordance with the findings of Doyle & Marth (1978a,b), Holmquist *et al.* (1983), Hagler *et al.* (1982) in corn and Altug *et al.* (1990) in figs.

More profound was the effect of temperature on mold growth and aflatoxin production. As can be deduced from Table 5.4 in samples having the same initial aflatoxin content (20 ng/g), low temperature restricted growth of *A. flavus* and production of aflatoxins. However, a reduction of the amount of aflatoxin present during the first month of storage occurred, indicating that some unknown factors may be degrading the aflatoxins. On the other hand, a temperature of 25°C (which is known to be a favourite for mold growth and aflatoxin production) seemed to enhance mold growth at the beginning of storage together with aflatoxin production (5-fold production of aflatoxins after the first month of storage compared to cold storage), but as moisture and a_w are progressively lowered, mold growth was suppressed and no more aflatoxin production occurred.

The effect of modified atmosphere packaging on growth of *A. flavus* and aflatoxin production is presented in Table 5.5. The vacuum here is used only for comparing reasons (in order to study the occurrence of aflatoxins during storage under the same atmospheric conditions as those used in Chapter 4). It is clear that, atmospheres rich in CO_2 suppress growth of *A. flavus* and aflatoxin production, while air enhanced aflatoxin production, but only during the first months of

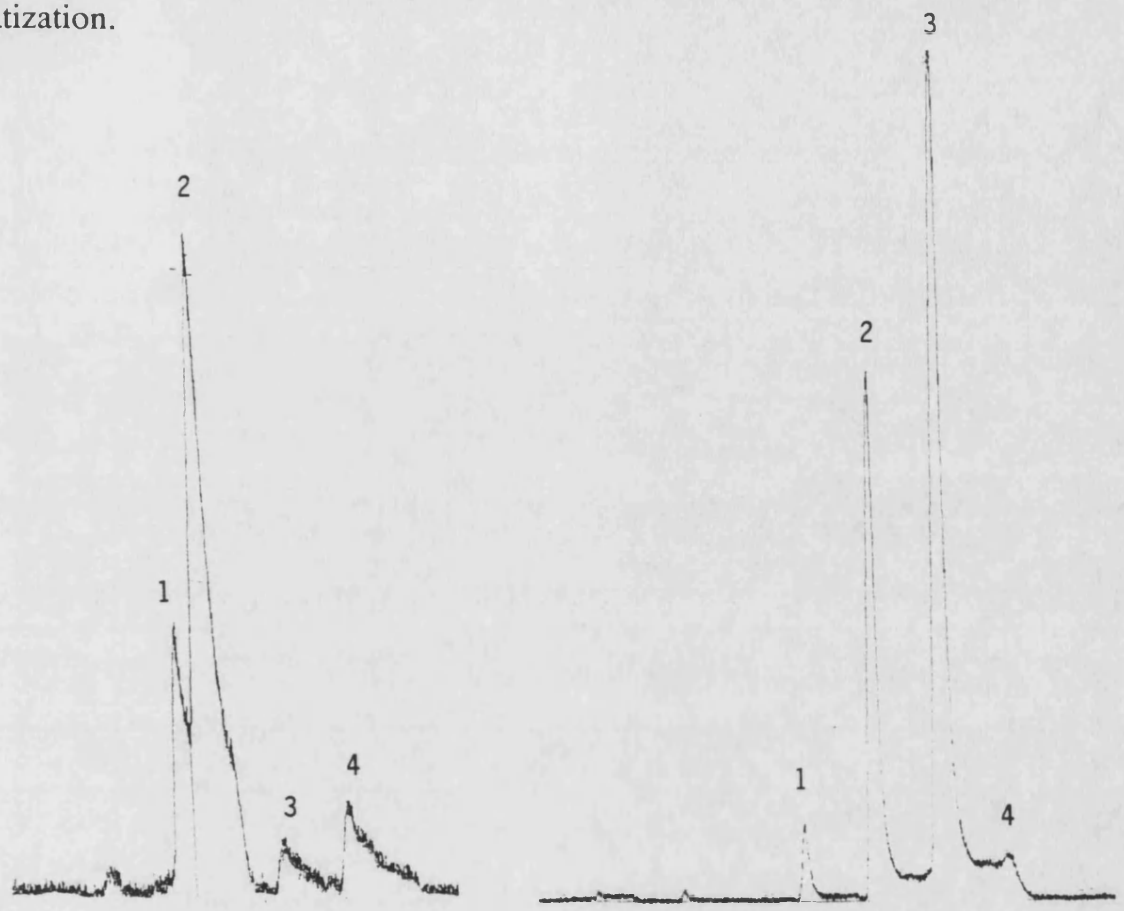
storage. After three months and till the 12th month of storage, the behavior of all samples was identical because the other storage conditions (a_w, moisture) did not support growth of mold and aflatoxin accumulation. These results agree with those of Landers *et al.* (1967) and Sanders *et al.* (1968) in peanuts, with those of Wilson & Jay (1975), Wilson *et al.* (1977) and Buchanan *et al.* (1985) on corn, peanuts and rice. Also the long term stability of aflatoxins in fig products shown by our experiments, agrees with analogous findings of Baur (1974) for peanuts.

It should be noted here that the control samples for all of the cases showed no signs of *Aspergillus*-like mold infection and also tested negative for aflatoxin, indicating that conditions of handling, processing and storage of figs may not be ideal for aflatoxin production.

High Performance Liquid Chromatography analysis of figs for aflatoxins

The typical profile of the reference aflatoxin standards analysed with HPLC with and without previous trifluoroacetic acid (TFA) derivatization is presented in Fig.5.4. It is clear that in underivatized samples (Fig. 5.4b) mostly aflatoxin B_1 and to a lesser extent G_1 (which are the most toxic) fluoresced less strongly than the derivatized samples and hence the identification of their peaks was difficult. When treated with trifluoroacetic acid, aflatoxins B_1 and G_1 were converted to their hemiacetals B_{2a} and G_{2a} which fluoresced strongly in polar media (Fig. 5.4a). Aflatoxins B_2 and G_2 do not react but are already highly fluorescent in aqueous mobile phases. The order in which aflatoxins were eluted was G_2, G_1, B_2 and B_1 for the non derivatized and G_{2a}, B_{2a}, G_2 and B_2 for the derivatized mixtures respectively as can be seen from Table 5.6. It is also notable that a slight shift in retention times occurred when aflatoxins were in mixture than when they were injected alone (Fig. 5.5a-e, Table 5.6). Those results are in agreement with the findings of Gregory & Manley (1981). In dried fig samples, the identification of the peaks of aflatoxins was achieved by using the reference aflatoxin substances (Fig. 5.5a-e) and also by adding a solution of the reference mixture to a sample extract (Fig. 5.6a-b). The variation in the solvent peak is due to the different scale

Fig. 5.4. HPLC analysis of reference aflatoxin mixture (B_1, B_2, G_1 and G_2) diluted in toluene-acetonitrile (98:2) a) with previous TFA derivatization and b) without TFA derivatization.



(a)

1: aflatoxin G_{2a}
2: aflatoxin B_{2a}
3: aflatoxin G_2
4: aflatoxin B_2

(b)

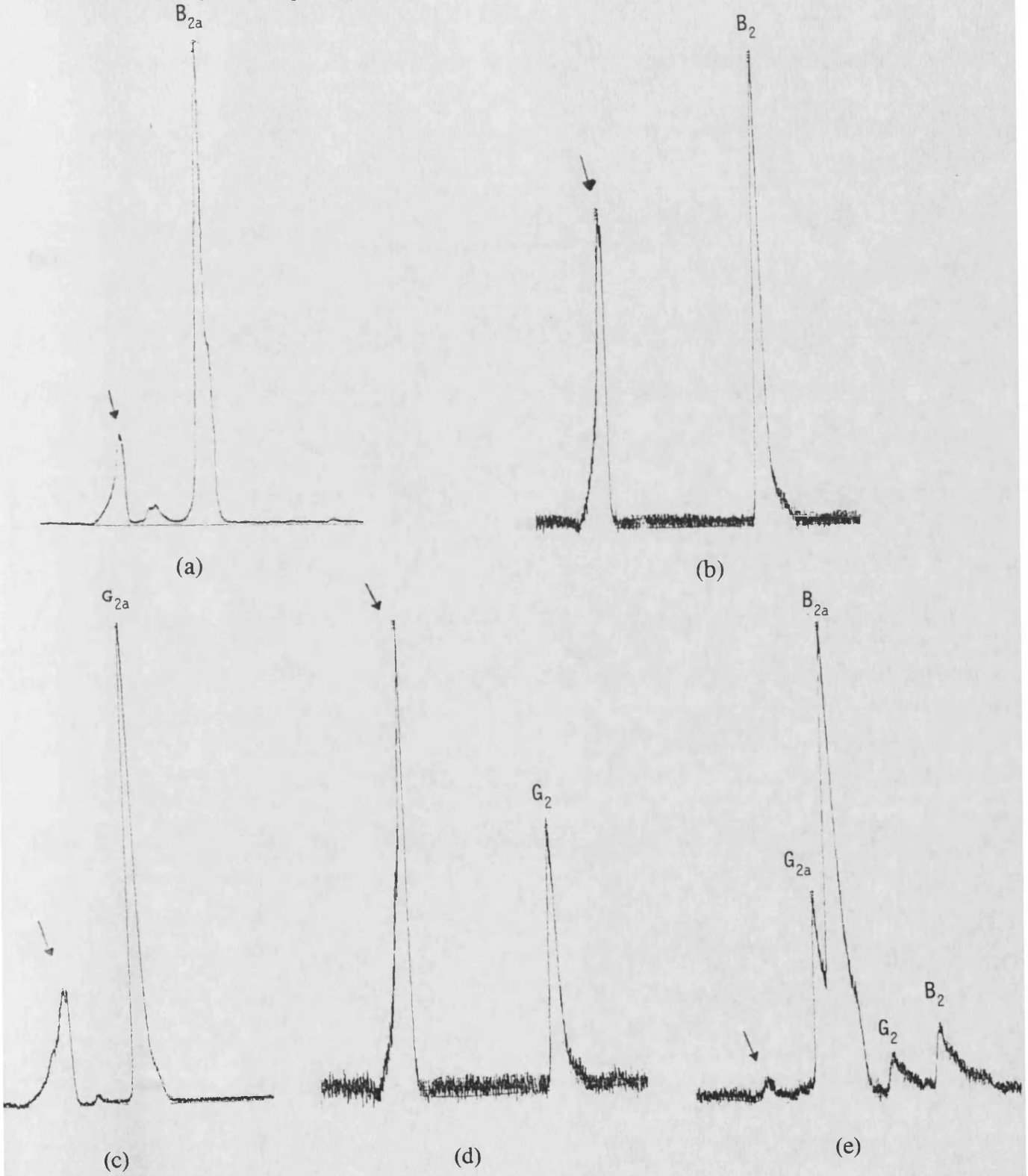
1: aflatoxin G_2
2: aflatoxin G_1
3: aflatoxin B_2
4: aflatoxin B_1

The concentration of aflatoxins in the reference mixture (a) was 0.5μg/5.05ml for B_1,G_1 and 0.1μg/5.05ml for B_2,G_2
The concentration of aflatoxins in the reference mixture (b) was 100 ng/g for B_1, G_1 and 20 ng/g for B_2, G_2

Table 5.6. Retention times for reference aflatoxins B_1, B_2, G_1 and G_2 with and without TFA derivatization, alone or in a mixture.

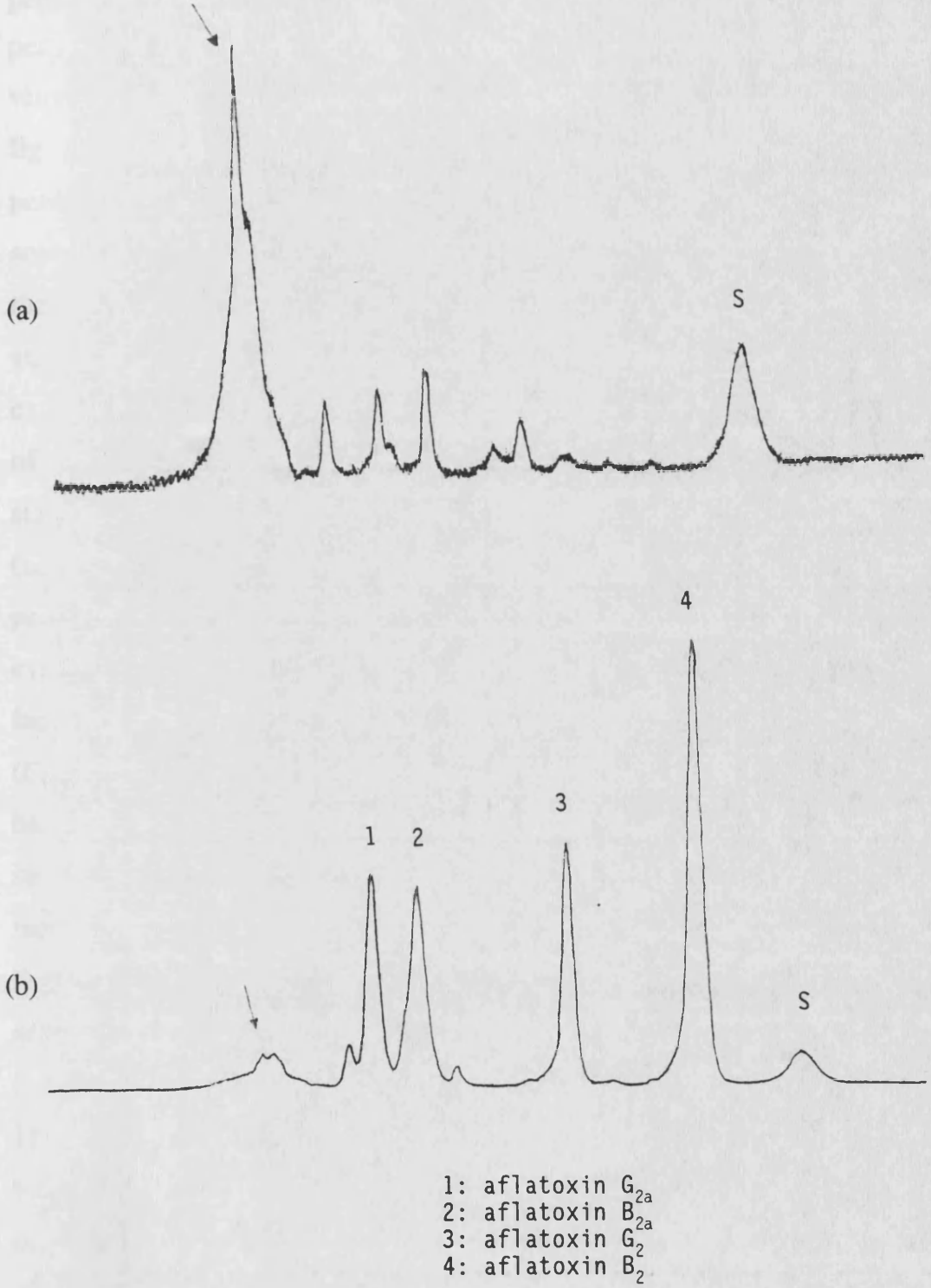
Name of compound	Retention times (minutes)			
	non derivatized alone	derivatized alone	non derivatized mixture	derivatized mixture
Aflatoxin B_1	10.77		9.65	
Aflatoxin B_{2a}		4.80-5.08		4.50-4.56
Aflatoxin B_2	8.90	8.33-8.60	8.22	7.72-7.79
Aflatoxin G_1	8.62		7.86	
Aflatoxin G_{2a}		4.21-4.34		4.01-4.02
Aflatoxin G_2	7.02	6.68-6.90	6.63	6.28-6.32

Fig. 5.5. HPLC analysis of reference aflatoxin compounds (TFA-treated) diluted in toluene-acetonitrile (98:2) a) aflatoxin B_1 (100 ng/g) b) aflatoxin B_2 (20 ng/g) c) aflatoxin G_1 (100 ng/g) d) aflatoxin G_2 (20 ng/g) e) mixture of B_1, B_2, G_1 and G_2 (concentration as given in p.158)



Peaks indicated by an arrow are possibly attributed to solvent

Fig. 5.6. HPLC analysis of sulphured dried figs a) aflatoxin-free sample and b) sample spiked with 5 ng/g of each of the aflatoxins B_1 and G_1 and 15 ng/g of each of the aflatoxins B_2 and G_2.



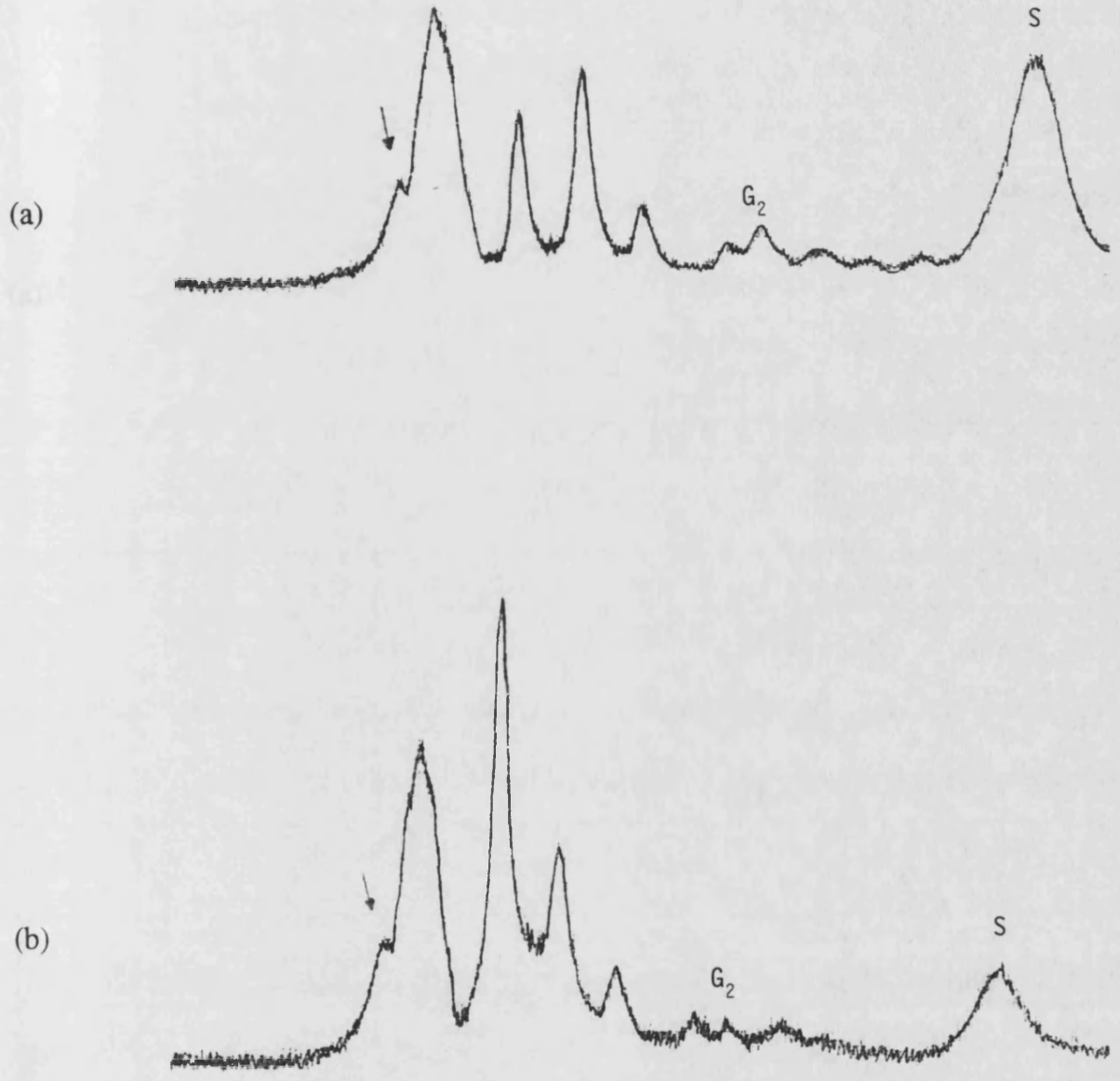
1: aflatoxin G_{2a}
2: aflatoxin B_{2a}
3: aflatoxin G_2
4: aflatoxin B_2

Peaks indicated by an arrow are possibly attributed to solvent

Peaks indicated by S are possibly related to SO_2

of the chromatographic profiles which was inevidable as aflatoxins B_{2a} and G_{2a} are highly fluorescent and give very high peaks, while B_2 and G_2 gave intermediate peaks. It is clear from those chromatograms that aflatoxins were not the only peaks, there were some other, but smaller peaks also (dependant on the attenuation-sensitivity used), which can be attributed to solvent and some other substances of fig which do not react with TFA but are highly fluorescent. An intense narrow peak (shown by an arrow) which eluted before the aflatoxin, was shown to be the solvent (TFA). The amount of aflatoxins B_2 and G_2 used for spiking was 3-fold than that of B_1 and G_1 based on the fact that peaks of B_2 and G_2 were relatively small in standard mixture solutions and thus might not be detected in fig sample extracts (where a lot of background interference is present). That is why the peaks of aflatoxins B_2 and G_2 are relatively high in derivatized fig extract compared to the standard mixture. A relatively big peak, shown at the end of chromatograms (named as peak S) was probably related to the SO_2, because in sulphured figs that peak was more intense than in the unsulphured ones (Fig. 5.7 a-b). Further evidence that led me to suspect that this peak was somehow related to SO_2 was the fact that a week after packaging, the intensity of that peak was not really reduced (Fig. 5.8a), but after a year of cold storage it was minimized (Fig. 5.8 b). It must be emphasized however that there is a lack of published work about the role of SO_2 in HPLC analysis of aflatoxins in sulphured dried figs or other dried fruits, thus more work needs to be done to establish the validity of the above contentions. From those chromatograms is also notable the increase throughout storage of aflatoxin G_2 which was found in some samples of sulphured dried figs analysed (the 1.1 ng/g of aflatoxin G_2 in the beginning of storage was increased to 5.2 ng/g after 12 months) and which is probably due to the fact that aflatoxin G_2 did not react with bisulphite at the beginning of storage. Hot washing, a treatment of the sulphured dried figs before packaging seemed to affect the chromatographic profile of sulphured dried figs as shown in Figures 5.9a and b. Hot washing enhanced the fluorescence of aflatoxin G_2 (1.7 ng/g compared to 0.9 ng/g in non-washed figs)

Fig. 5.7. HPLC analysis of a) sulphured and b) non sulphured dried figs one week after packaging.

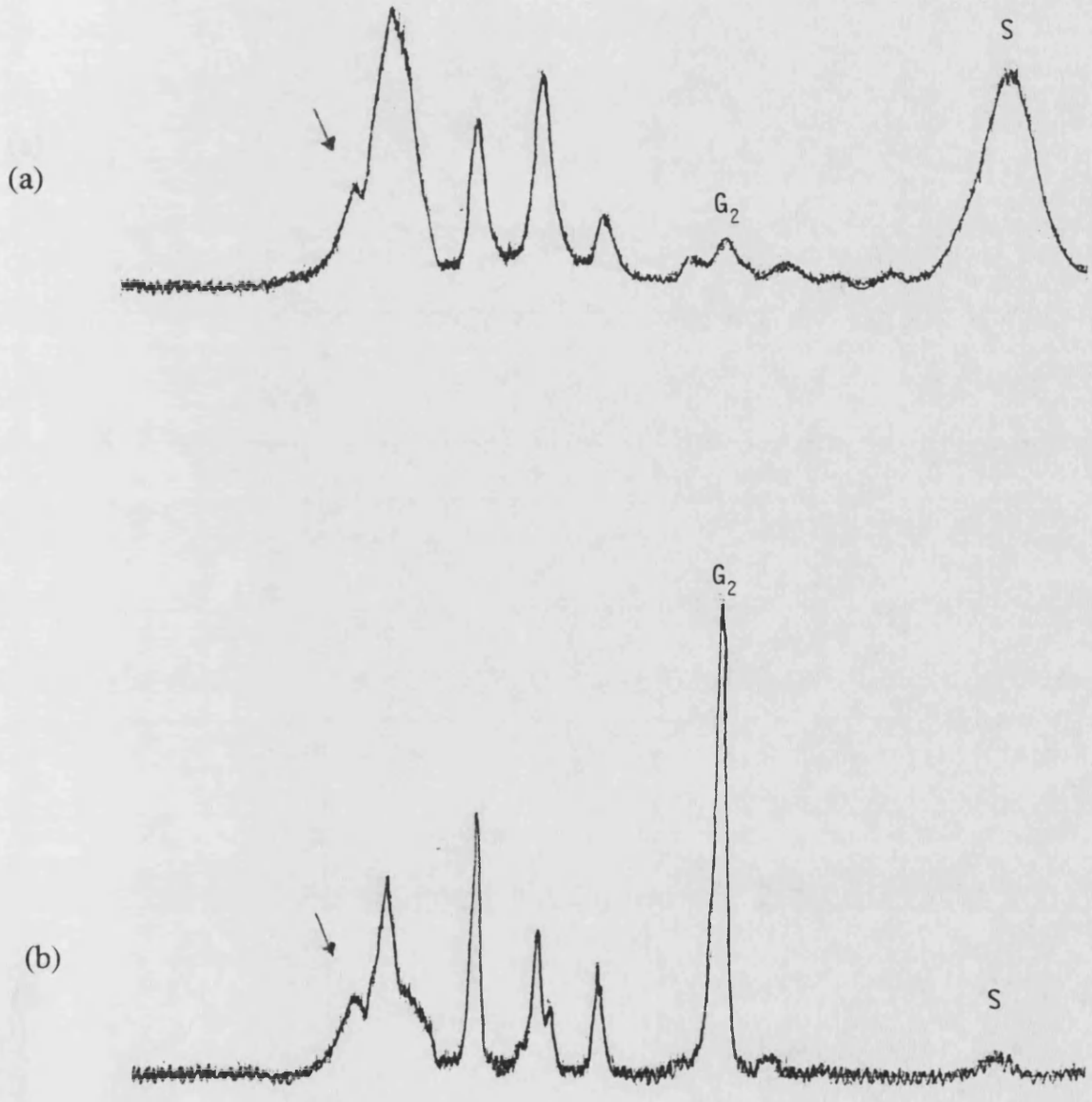


Peaks indicated by an arrow are possibly attributed to solvent

Peaks indicated by S are possibly related to SO_2

The concentration of aflatoxin G_2 was calculated as described in materials and methods pp.68

Fig. 5.8. HPLC analysis of sulphured dried figs a) a week after packaging b) after 12 months of storage at 4°C.

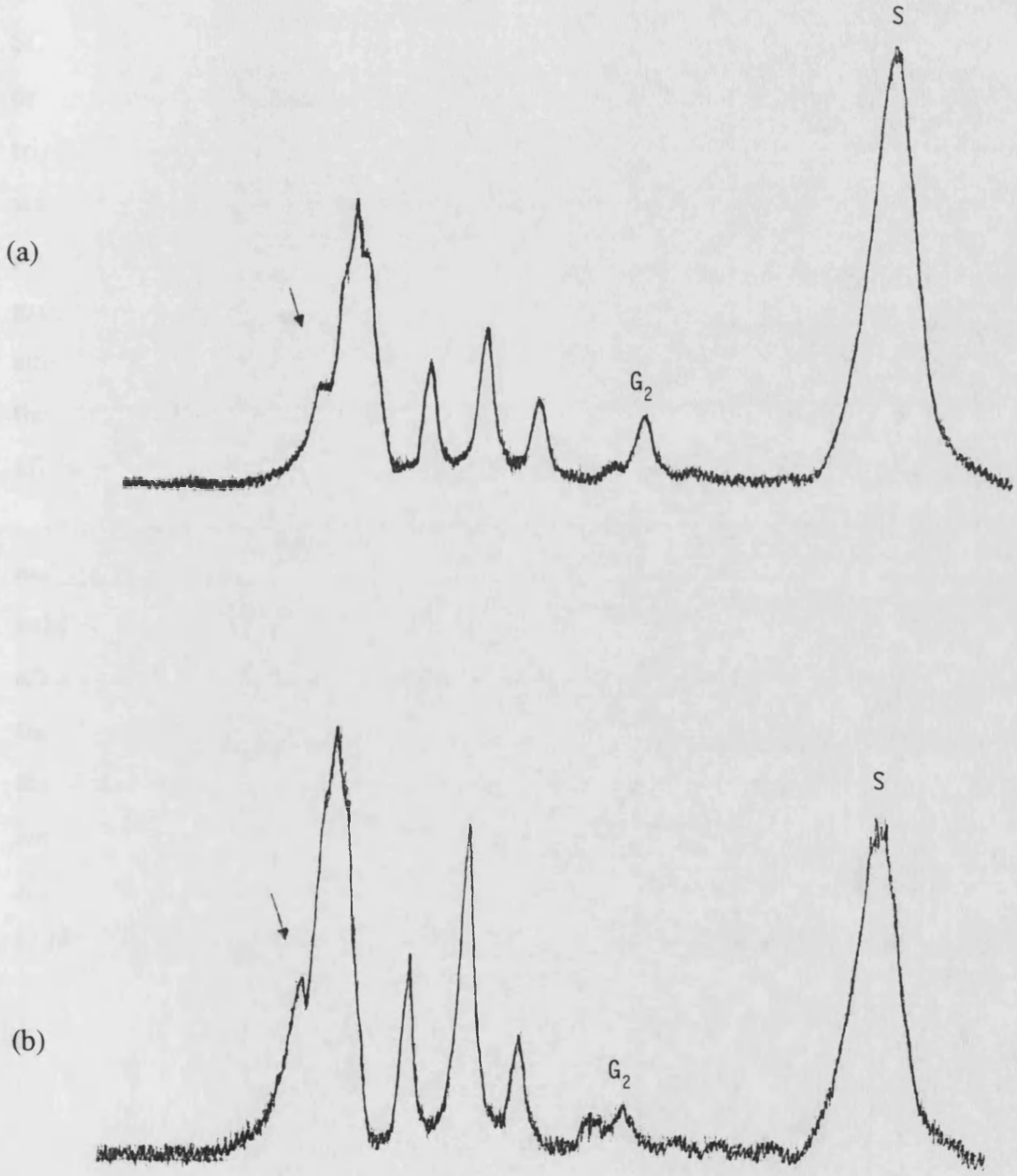


Peaks indicated by an arrow are possibly attributed to solvent

Peaks indicated by S are possibly related to SO_2

The concentration of aflatoxin G_2 was calculated as described in materials and methods pp.68

Fig. 5.9. HPLC analysis of sulphured dried figs a) with and b) without previous hot washing.



Peaks indicated by an arrow are possibly attributed to solvent

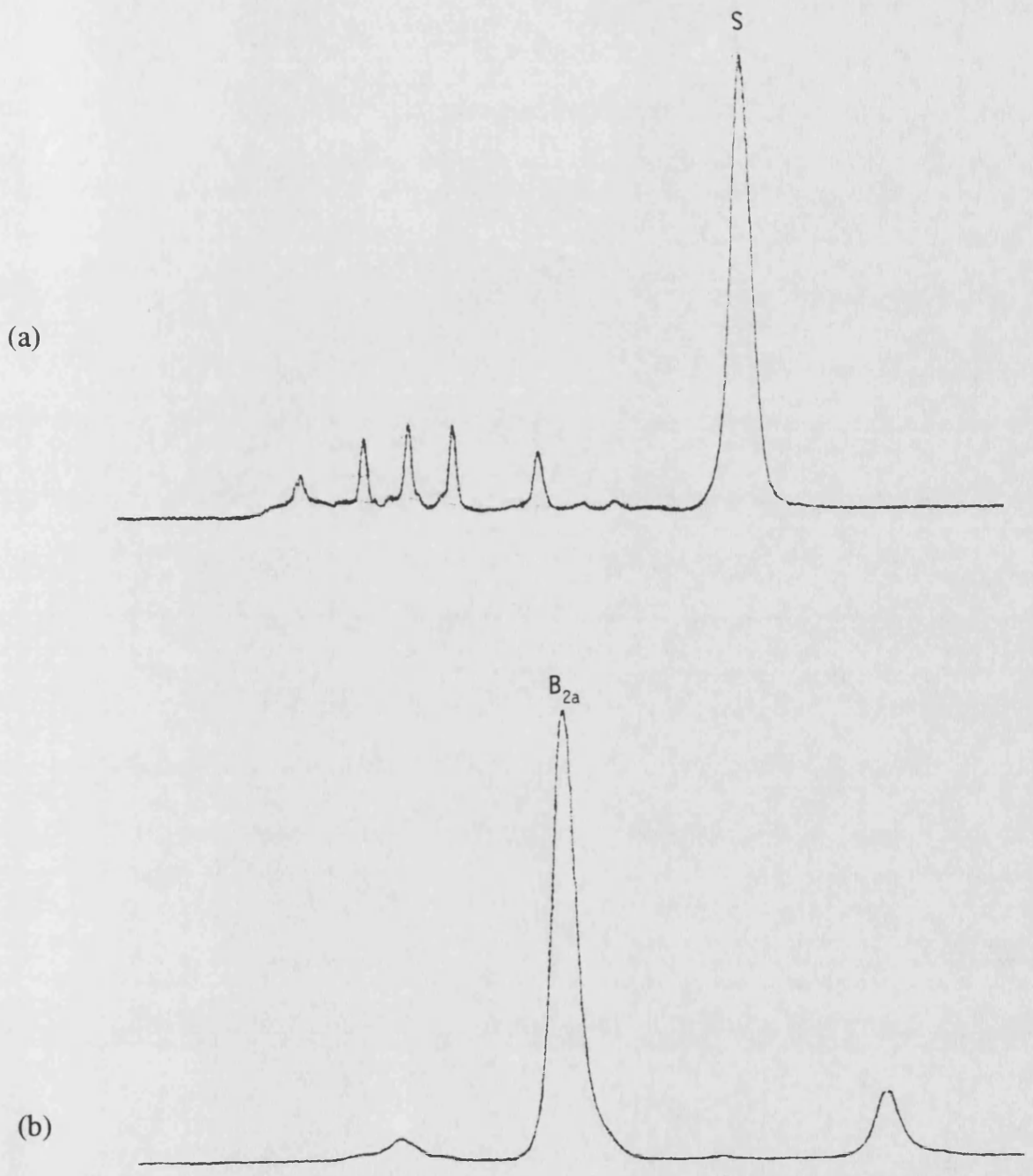
Peaks indicated by S are possibly related to SO_2

The concentration of aflatoxin G_2 was calculated as described in materials and methods pp.68

and reduced intensity of the other fluorescent products, but surprisingly increased also the peak that we have related to SO_2, in spite of the fact that according to our previous findings (Chapter 3, Fig. 3.12) the SO_2 content of figs was lowered by hot washing. A possible reason for the lack of agreement could be that either some SO_2 residues were left in column from previous analyses which enhanced the peak, or some more free SO_2 was liberated by hot washing which furtherly reacted with trifluoroacetic acid (In Chapter 3, the amount of free SO_2 before and after hot washing had not been determined, only the total SO_2 was measured). It should be pointed out here that the presence of aflatoxin G_2 in some fig samples was not of a great significance as only 3 samples out of 43 tested were found contaminated with small amounts of aflatoxin G_2 (0.9-1.7 ng/g) and those are the ones presented in the figures. It must also be kept in mind that aflatoxin G_2 is less toxic than aflatoxins B_1 and G_1.

In Figures 5.10 a-b and 5.11 the chromatographic profiles of extracts from naturally contaminated peanuts, containing 23.1 ng/g aflatoxin B_1 (Fig. 5.10b) and sultana raisins, containing 0.9 ng/g aflatoxin B_1, 24.9 ng/g aflatoxin B_2, 1.7 ng/g aflatoxin G_1 and 1.4 ng/g aflatoxin G_2 (Fig.5.11) are presented in comparison with the profile of the sulphured non-contaminated fig extract (Fig. 5.10a). It is evident that dried fig samples showed a high background interference compared to peanut and raisin extracts. The difficulty of dried figs as samples for HPLC analysis was also observed by MAFF (Food Science Laboratory 1989) and Wilson & Romer (1991).

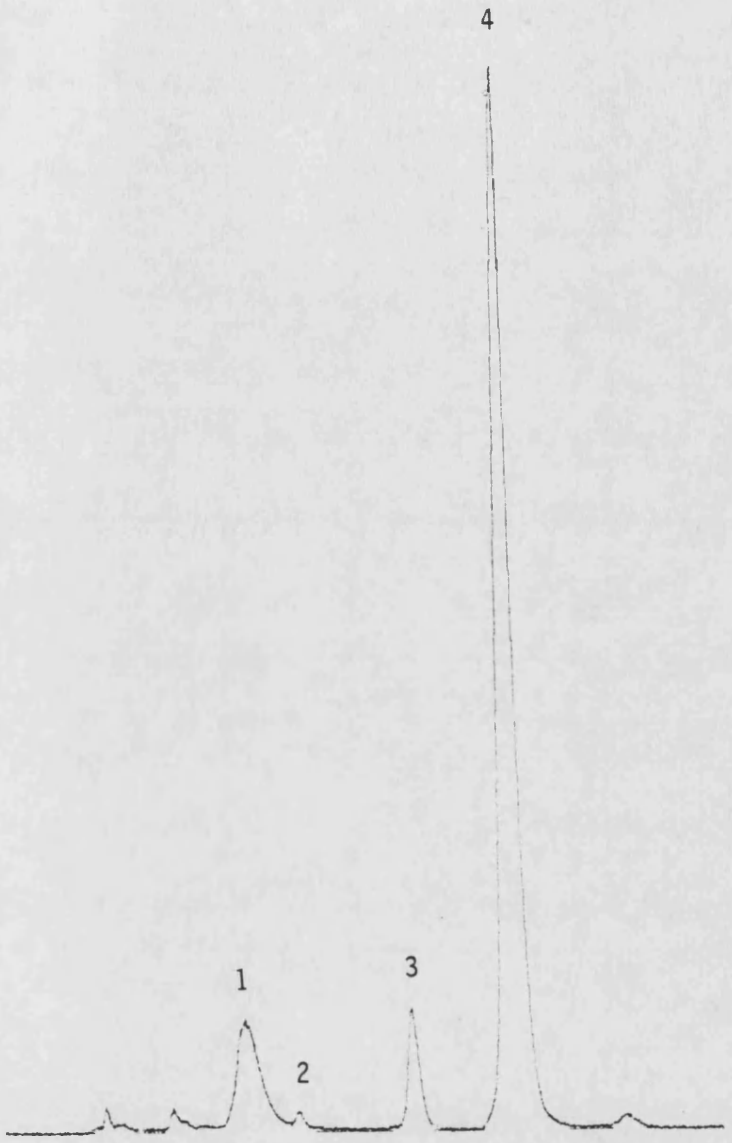
Fig. 5.10. HPLC analysis of a) sulphured dried figs b) naturally contaminated peanuts.



Peak indicated by S is possibly related to SO_2

The concentration of aflatoxin B_{2a} was calculated as described in materials and methods pp.68

Fig. 5.11. HPLC analysis of naturally contaminated sultana raisins.



1: aflatoxin G_{2a}
2: aflatoxin B_{2a}
3: aflatoxin G_2
4: aflatoxin B_2

The concentration of aflatoxins was calculated as described in materials and methods pp.68

Discussion

It is well known that the presence of aflatoxigenic fungi in foods might signify the potential for aflatoxin production within a substrate under conditions of optimum temperature and moisture (Diener & Davis 1967; Golumbic & Kulik 1969; Joffe & Lisker 1969; Northolt *et al.* 1976; Park & Bullerman 1983). Therefore, studies continue to be directed towards surveys of the occurrence of *A. flavus* and aflatoxin production in different commodities.

Contamination of figs especially from Turkey, was recently recognized by several authors (Steering group on Food Surveillance 1988; Akerstrand & Moller 1989; Gelosa 1990; Boyacioglu & Gönül 1990; Sharman *et al.* 1991) indicating that figs are a good substrate for *A. flavus* growth and aflatoxin production as a fruit rich in carbohydrates. Thus, it was important to examine if the Greek figs and specially the sulphured ones are susceptible to mold infection and aflatoxin production. It was evident in this part of my study that Greek figs if improperly handled, are also a good substrate for mold growth. However, the methods of handling, processing and storing that we have suggested to producers (picking figs from the tree, sulphuring procedure) ensures the figs are free from contamination but it does not give total protection of the crop as proved by the fact that some of our control samples showed mold contamination. Our experiments though showed clearly that none of those samples was infected by toxigenic *Aspergillus*.

The mechanism of natural infection of figs by *A. flavus* remains unclear. According to Buchanan *et al.* (1975) figs are very susceptible to infection by conidia of *A. flavus* on exterior of fruit surfaces as well as by conidia carried into the interior of the fruit by insects, when ripe enough to be eaten out of hand. This was to some extend exemplified by the results of this part of my study also. Spores dusted on the surface of highly susceptible fruits, were able to infect and colonize the fruits, suggesting that conidia of *A. flavus* are capable of penetrating the fruit

skin rather than requiring a wound for entry. Several alternative possibilities exist, however, which might have permitted a wound pathogen to enter fruit readily. Minute wounds might have been present in the fruit skin. The foraging of microscopic animals, such as insects or mites, might have caused wounds not readily visible to the naked eye. Or, sufficient fruit tissue may have been present on the fruit surface to permit some fungal colonization before penetration, an event often facilitating penetration. However, Gilbert (1989) suggested that figs were infected when falling into the ground. It is well known that incidence of infection, growth of mold and aflatoxin production is influenced by many factors such as inoculum size, pH, moisture, temperature of storage, presence of inhibitory agents etc (Rabie & Smalley 1965; Schroeder & Hein 1967; Schindler *et al.* 1967; Van Walbeek *et al.* 1969; Northolt *et al.* 1977; Sharma *et al.* 1980; Yousef & Marth 1981). In my results it was notable (Tables 5.3-5.5) that besides the quite big inoculum size, a small aflatoxin production occurred (10-50 ppb of total aflatoxins) which is probably due to the effect of sun radiation, sulphuring and blanching processes which are known to degrade aflatoxins (Samarajeewa *et al.* 1990; Tabata *et al.* 1992) as well as to possible microbial interaction of competing microflora, which could either convert aflatoxin B_1 to aflatoxicol (as *A.niger, Rhizopus,* non aflatoxin producing *A.flavus* do; Nakazato *et al.* 1990) or inhibit growth of *A.flavus* and suppress aflatoxin production (as *A.niger, A.ochraeus, Alternaria* spp. do; Roy & Chourasia 1990).

In recent years, a number of reliable mycological methods for detection and identification of aflatoxigenic fungi in foods have become available (Hara *et al.* 1974; Lin & Dianese 1976; Wicklow *et al.* 1981; Pitt *et al.* 1983; Lenovich *et al.* 1986). Our studies suggested that for laboratories with minimum equipment (as the Agricultural Cooperation of Fig producers at Kymi) and a need for results in a minimum time, the aflatoxigenic fungi detection and identiflcation maybe divided into 3 steps, giving that way a satisfactory monitoring of the crop:

1. Rapid presumptive tests on a differential culture medium - *A. flavus* and *parasiticus* agar (AFPA) - identify samples that may contain the suspected aflatoxigenic fungi.
2. Rapid screening procedures on Aflatoxin producing Ability (APA) medium measure the ability of the suspected aflatoxigenic fungi to produce aflatoxin.
3. Qualitative and semiquantitative methods to determine the total amount of aflatoxin produced by the aflatoxigenic *Aspergillus* (total aflatoxin test kit of Biocode).

The method of Steiner *et al.* (1988) suggesting a screening of figs under U.V. light for bright greenish yellow fluorescence was good to identify contaminated individual fruits and therefore to "clean" large lots of dried figs before retail distribution. However, from our study it can be concluded that it is not a reliable method for aflatoxin detection in contaminated samples. Figs showing fluorescence inside the fruit would be missed, as would any fruit where the fluorescence had been washed off, or lost through long exposure to sunlight as Marsh *et al.* (1969) showed in cotton and Steiner *et al.* (1988) in figs. Moreover, "sugared" figs may give false positive BGY fluorescence and indication of aflatoxin presence, as many of our figs giving BGY fluorescence did not contain aflatoxins when further analyzed (Table 5.2).

The effect of bisulfite and sulphite on degradation of aflatoxin was studied by several workers (Doyle & Marth 1978a,b; Walther *et al.* 1983; Hagler *et al.* 1983; Yagen *et al.* 1989). It is known though (Joslyn & Braverman 1954; Roberts & McWeeny 1972) that fruits may contain sugars, organic acids and other organic compounds which also may react with bisulphite. This could reduce the aflatoxin-degrading effects of bisulphite. Also as suggested by Tabata *et al.* (1992) a protective effect is exerted by reducing saccharides (such as glucose), proteins and aminoacids to aflatoxin containing in food against degradation. In a recent study,

Altug *et al.* (1990) investigated the application of bisulphite treatment as described to degrade aflatoxins, in dried figs, in coordination with other factors (temperature, ultra-violet radiation and H_2O_2) and he found that 28.2% of the toxin added to dried figs was degraded in 72 hours at 25°C when treated with 1% sodium bisulphite. Heating samples at 45-65°C for 1 hour caused up to 68.4% of the toxin B_1 to be degraded and ultra-violet radiation degraded 45.7% of aflatoxin B_1 in fig samples. Our studies revealed that the amounts of SO_2 contained in dried figs (average 1400 ppm) at the beginning of storage are adequate not only to degrade aflatoxin B_1 and G_1 but, the most important, to inhibit the growth of aflatoxigenic *Aspergillus* (Table 5.3). However, as the limiting factors associated with mold growth and aflatoxin production are no doubt altered as the product proceeds from the field to the market (fresh, dried, stored) the likelihood of aflatoxin occurrence in sulphured dried figs (provided conductive conditions) should not be dismissed.

In recent years, high performance liquid chromatography (HPLC) has been widely applied to the separation and determination of aflatoxins in foods (Hunt *et al.* 1978; Beebe 1978; Davis & Diener 1980; Gregory & Manley 1981; Wilson & Romer 1991), specially in peanuts and products thereof (Tarter *et al.* 1984; Dorner & Cole 1989; Azer & Cooper 1991). Our work was the first dealing with the interference of sulphuring on HPLC analyses for aflatoxins in dried figs. It should be noted here that the HPLC method used was developed on the base of the available instruments and the extraction methods used officially for aflatoxins in Greece. The results of this part of my study revealed that sulphured dried figs can be contaminated by aflatoxin G_2 (which is not reacting with bisulphite). However, dried fig samples offered analytical problems such as large general background interferences due to other fluorescent substances present and therefore our results suggest the necessity of a clean-up of sample extracts before the HPLC analysis.

RECENT DEVELOPMENTS IN FOOD MYCOLOGY AND MYCOTOXINS IN FOODS

Introduction

The occurence, biosynthesis, toxicology, analysis and significance of aflatoxins and aflatoxigenic fungi in foods have been reviewed in chapter 1 (pp.33-45) of this work. However, in recent years there is a considerable primary literature dealing with the mycotoxins in foods as some new mycotoxins have been found - fumonisin, discovered in 1988 (Nelson *et al* 1993) and a lot of work has been done on the spoilage fungi active on stored commodities. In the following it is hoped to provide further information about the recent developments in food mycology as well as in mycotoxigenic fungi and their mycotoxins that had not been described in Chapter 1.

The ability of filamentous fungi to actively grow and metabolize at relatively low water activities, coupled with their mode of vegetative growth, enables them to grow over and through solid substrates. They are able to absorb low molecular weight nutrients and many also produce, and secrete, secondary metabolites, which are also relatively low molecular weight compounds but not obviously associated with the process of growth and primary metabolism (Bushell 1989). There is a continuing debate about the nature of secondary metabolism and the role of secondary metabolites in the biology of the producing organisms (Vining 1992) but many of these secondary metabolites do have biological activity and may be toxic to microorganisms (antibiotics), plants (phytotoxins) or animal (mycotoxins).

Many moulds are important in the biodegradation of complex materials of plant and animal origin and contribute to the cycling of elements such as carbon, nitrogen and phosphorus in the biosphere but this same activity also makes them important agents in the biodeterioration of foods, feeds and the raw materials used in their manufacture.

Postharvest, stored commodities such as cereals, oilseeds and animal feeds, are especially susceptible if stored at high water activities and warm temperatures. It is in these commodities, and under those conditions, of poor storage, that the highest levels of mycotoxins are often experienced. Many fungi can also interact with living plants as pathogens, symbionts or endophytes and during any of these processes, mycotoxins may be secreted into the living tissue of plants before harvest, or into animal pastures, consumption of which may then cause illness, or even death (Moss 1995a).

"It is perhaps worth noting that, in terms of acute toxicity, even the most poisonous of the mycotoxins commonly encountered in food are a factor of about a million times less toxic than the most virulent of the bolutinum toxins and a factor of a thousand times less toxic than many algal toxins (Table 1). Indeed the number of cases of confirmed acute mycotoxin poisoning in humans during the last decades is very small compared with the number made ill by food poisoning bacteria such as *Salmonella* and infections diseases. It is, however, the possibility of long term chronic toxicity which is of special concern because several of those mould metabolites are known to be teratogenic, carcinogenic, oestrogenic or immunosuppressive and their presence in foods may have more subtle effects on human health especially in the developing countries of the tropics where people may use badly stored plant products". Quoted directly from Moss (1996).

Mycotoxigenic fungi and their toxins

The nature and extent of toxigenic fungal contamination will determine the presence or absence of mycotoxins in the product. While the identification of contaminating fungi can be of diagnostic value in outbreaks of mycotoxicosis, positive conclusions may only be derived by extraction and identification of the suspected toxin(s) since:

Table 1. Acute LD_{50} values for microbial toxins. Where a range is recorded in the literature the lower figure is given.

Toxin	LD_{50} ($mg.kg^{-1}$)	Producing organism
Aflatoxin B_1	5.5 (oral, male rat)	*Aspergillus flavus*
Aflatoxin B_1	17.9(oral, female rat)	*Aspergillus flavus*
Citrinin	35 (i.p., rat)	*Aspergillus flavus*
Deoxynivalenol	46 (oral, mouse)	*Fusarium graminearum*
Fumonisin B	?	*Fusarium moniliforme*
Ochratoxin A	28 (oral,male rat)	*Penicillium verrucosum*
Ochratoxin A	21.4(oral, female rat)	*Penicillium verrucosum*
Patulin	35 (oral, mouse)	*Penicillium expansum*
Penitrem A	1.1 (i.p., mouse)	*Penicillium aurantiogriseum*
PR toxin	58 (oral, mouse)	*Penicillium roquefortii*
Rubratoxin B	120 (oral, mouse)	*Penicillium purpurogenum*
T-2 toxin	5.2 (oral, rat)	*Fusarium sporotrichioides*

Adapted from Moss, M.O (1996)

1. The presence of the fungus is no assurance that it was producing the toxin (as it was shown by my experiments for aflatoxins in figs, p.149)

2. A given toxin may persist in product when the fungus is no longer present (it was also shown in my experiments p.153-154)

3. A given fungus may be capable of producing more than one toxin.

4. A given toxin may be produced by different genera of fungi.

Table 2 outlines the main possible routes for mycotoxin entry into human and animal foods.

The main groups of toxigenic fungi and their mycotoxins will now be outlined.

The *Aspergillus* Mycotoxins

The mycotoxins produced by the aspergilli which are considered to present a potential serious concern to animal and human health are the aflatoxins sterigmatocystin, a-cyclopiazonic acid and ochratoxin A.

Aflatoxin is still a problem in many commodities. This is the fourth decade of research on aflatoxin contamination of food and feed and despite the expenditure of a large research effort worldwide, aflatoxin continues to be a major problem (Hussain & Vojir 1993; Tabata *et al.* 1993; Doster & Michailides 1994; Viquez *et al.* 1994). In addition to *A. flavus* and *A. parasiticus*, *A. nomius* is also found to produce aflatoxins. These moulds occur in warmer parts of the world and aflatoxins may be produced in a wide range of tropical and subtropical food commodities, such as figs, tree nuts and cereals, but the most important crops are maize and ground nuts. The highest levels of contamination have been recorded recently in maize, peanuts, brazil nuts, pistachio nuts, cottonseed and copra (Cvetnic 1994; Resnik *et al.* 1996). Lower levels of contamination

Table 2. Possible routes for mycotoxin contamination of human and animal foods.

Mould damaged foodstuffs
(a) Agricultural products e.g cereals oilseeds (groundnuts) fruits vegetables
(b) Consumer foods (secondary infections) Compounded animal feeds (secondary infections)
Residues in animal tissues and animal products e.g
milk dairy produce meat
Mould-ripened foods, e.g.
cheeses fermented meat products oriental fermentations
Fermented products, e.g.
microbial proteins enzymes food additives such as vitamins

have been observed in recent years with almonds, pecans, walnuts, raisins, spices and figs (Masson & Meier 1988; MAFF 1994; Sharman *et al.* 1994). Many crops show resistance or only moderate susceptibility to aflatoxin contamination in the field viz: soybeans, grain sorghum, wheat, oats, barley and rice (Abdel-Mallek *et al.* 1994; Atawodi *et al.* 1994). Although initially considered as a post-harvest problem of poorly stored commodities, contamination of important crops can also occur during active growth of the plant in the field. The complex ecology of *A. flavus* and *A.parasiticus* has been reviewed by Diener *et al.* (1987) and it is now appreciated that *A. parasiticus* is well adapted to a soil environment and is predominent in peanuts, whereas *A. flavus* seems to be adapted to active development on the aerial parts of plants, such as leaves and flowers, and is dominant on maize, cottonseed and tree nuts.

Many recent studies have demonstrated that environmental and biological factors influence the production of aflatoxins (Moss 1989, Moss *et al.* 1990). Other microorganisms growing with *A. flavus* or *A. parasiticus* can influence the final yield of aflatoxin either negatively as lactic acid bacteria (Gourama & Bullerman 1995a, b) or positively as *A. niger* (Choudhary & Sinha 1993). The ecology and mycotoxigenic capability of *Aspergillus* may also be influenced by the presence of biocides (Moss & Frank 1985a; Badii *et al.* 1986; Moss & Frank 1987; Badii & Moss 1988).

During the latter part of 1988 there were reports from several European countries that high levels of aflatoxin were been identified in some samples of Turkish dried figs. Of the samples of whole dried figs analysed, about 78% contained total aflatoxins above 4µg/Kg and 11% contained aflatoxins above 10µg/Kg. The maximum level found was 427µg/Kg (Figure 1). From that time on, aflatoxins were found in dried figs elsewere, as well as in fig pastes (Hussain & Vojir 1993; Steiner *et al.* 1993; Finoli *et al.* 1994). None of the above investigators have found aflatoxin G_2 in dried fig samples, most of them being contaminated with aflatoxins B_1 and G_1. Those findings are in contrast with

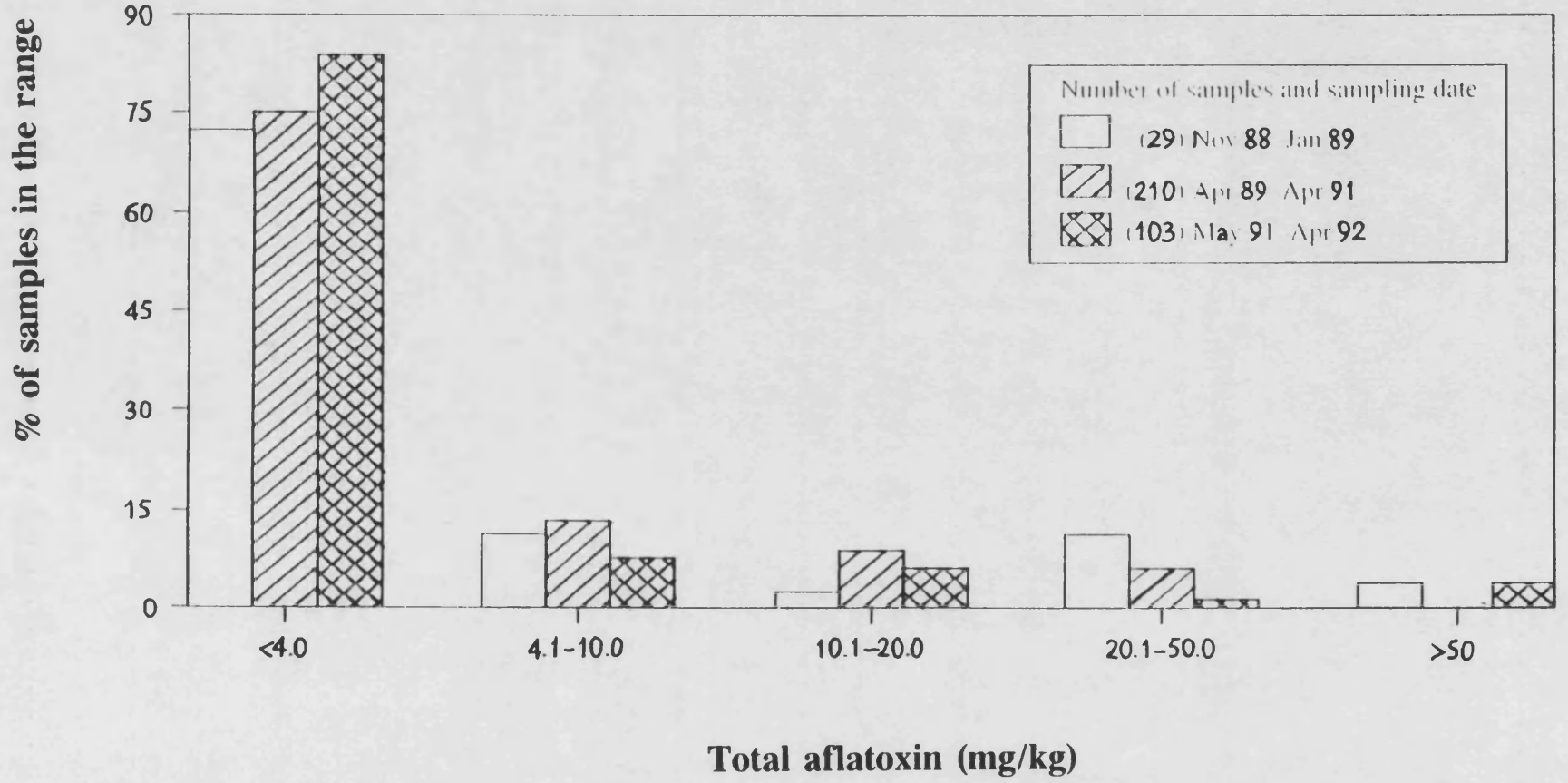


Figure 1. Distribution of total aflatoxin in samples of whole dried figs collected between November 1988 and April 1992.

Adapted from MAFF Food Surveillance Paper (1993)

our results (p. 162) according to which the presence of aflatoxin G_2 was detected in one fig sample. It is probable that our sample was not in fact contaminated by aflatoxin G_2 and the positive result was due to traces left in the HPLC column, from previous analyses.

Figs containing high concentrations of aflatoxins can generally be detected on the basis of their BGY fluorescence under UV light (Steiner *et al.* 1988). Wenk *et al.*(1994) but our study also (p. 152) showed that non fluorescent figs can as well contain appreciable amounts of aflatoxins, a finding that has not so far been taken into account in inspections of dried figs in many countries.

The effects of harvesting method, drying pretreatment and method of drying on contamination of figs with aflatoxins were recently investigated by Ozay *et al.* (1995). Aflatoxin B was detected in one sample which was harvested by the classic turkish method (waiting until fruits fall to the ground) and dried by exposure in sunlight. It was suggested that harvesting of fruits by hand, pretreatment with a dipping solution and drying as soon as possible by a solar drier was effective in reducing mould and aflatoxin contamination of figs. That was also our suggestion as a result of our experiments.

As it concerns the occurrence of aflatoxigenic fungi, a very recent research of Doster *et al.* (1996) showed that although *A. parasiticus* was rarer in fig fruit than *A. flavus*, it was more frequent in orchard soil than *A. flavus*. High levels of total aflatoxin (>100 ng/g) were detected in 83% of the figs infected by *A. parasiticus*, but in only 32% of the figs infected by *A. flavus*. Other *Aspergillus* species were also found as well (*A. niger, A. japonicus, A. ochraceus, A. melleus* and *A. alliaceus*) as natural contaminants in fig fruits but only 40% of the figs with these fungi had more than a trace of ochratoxin. The above are in accordance with our findings for dried figs of Kymi (p. 146-149) which were infected naturally only by *A. flavus* but did not produce aflatoxins whereas the figs

inoculated by *A. parasiticus* strain proved to be a good substrate for the production of aflatoxins.

Here, it should be pointed out that the DG-18 medium used for the enumeration of fungi in dried figs in our experiments (p. 132) even suggested by other researchers Pitt (1989a) is a suitable medium for the enumeration of all except extreme xerophilic fungi, as its a_w is around 0.955. Kocabas *et al.* (1993) suggested other media suitable for enumeration of fungi and total fungal flora from dried figs such as Dichloran Rose Bengal Chloramphenicol Agar (DRBC), Rose Bengal Streptomycin Agar (RBSA) etc. Other researchers (Tapia de Daza & Beuchat 1992) have suggested a modified dichloran - glycerol (DG18) agar by adding ionic and non-ionic surfactants or fungicides (Triton X-301 or iprodione) but all the above media are no better selective for xerophilic fungi than DG18.

The ochratoxins are a group of structurally related mycotoxins from which ochratoxin A is by far the most common. While *A. ochraceus* is the main ochratoxin producer within *Aspergillus* species, it is also widely produced from *Penicillium verrucosum.*

Sterigmatocystin is another toxic metabolite closely related to the aflatoxins and has been isolated from a wide range of aspergilli in particular, *A. versicolor, A. glaucus* and *A. nidulans*. Chemically, it is characterised by a xanthone moiety fused to a dihydrodifuran or tetrahydrofuran moiety it is acutely toxic and highly carcinogenic but less so than aflatoxin B_1.

The *Fusarium* Mycotoxins

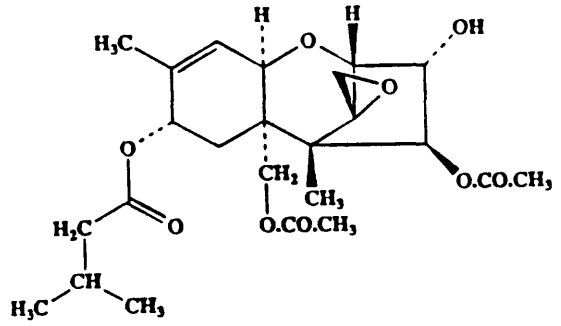
The major mycotoxins produced by the *Fusarium* species are trichothecenes, fumonisins, fusarin C, moniliformin and zearalenone.

Approximately 24 species of *Fusarium* are now known to produce trichothecenes, the most important of those being *F. sporotrichioides* (Moss & Frank 1988). The trichothecenes are the most chemically diverse of all the mycotoxins. Of over 150 known trichothecenes, only a few appear to be of importance with respect to their actual presence in crops intented for human or animal use, the most common beeing T-2, DON and nivalenol. While the other trichothecenes have been demonstrated under laboratory conditions, there is little evidence to indicate that they occur to any extent under natural conditions (Scott 1989).

Almost all of the fungal species that produce trichothecenes are plant pathogens or attenuated plant pathogens and it is possible that these compounds play a part in the pathogenicity of the fungal species by attacking specific targets in the host defences. Trichothecenes are highly toxic at the subcellular, cellular and organic system level. The common symptoms of trichothecene toxicity are dermal irritation, haemorrhagic lesions, pathological changes in the haemopoietic organs and depression of immune response.

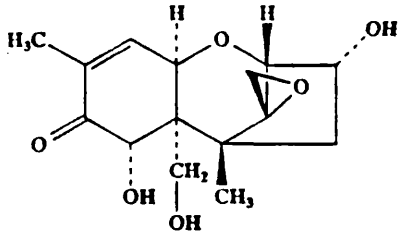
Although fortunately not frequently found in foods, one of the most toxic of the *Fusarium* trichothecenes is T-2 toxin (Fig. 2a). This toxin is thought to have contributed to the epidemiology of the terrible outbreaks of alimentary toxic aleukia in Russia at the beginning of this century. Deoxynivalenol (DON) also known as vomitoxin (Fig. 2b), is a far more common, but much less toxic, trichothecene and is produced by species such as *F. graminearum* and *F. culmorum*. Despite the low acute toxicity of DON, it is a potent antifeedant and induces a vomiting response in the pig at very low concentrations.

The most recently characterized mycotoxins of any major significance in human health are the fumonisins. This increasingly important new group of mycotoxins was first isolated in 1988 from cultures of *Fusarium moniliforme* (Gelderblom *et al.* 1988; Rheeder *et al.* 1992) a species of worldwide significance. More recently, *F. proliferatum* and *F. nygamai* have also been shown to produce these toxins. The basic chemical structures have been shown to be the diester of propane-1,2,3 -tricarboxylic



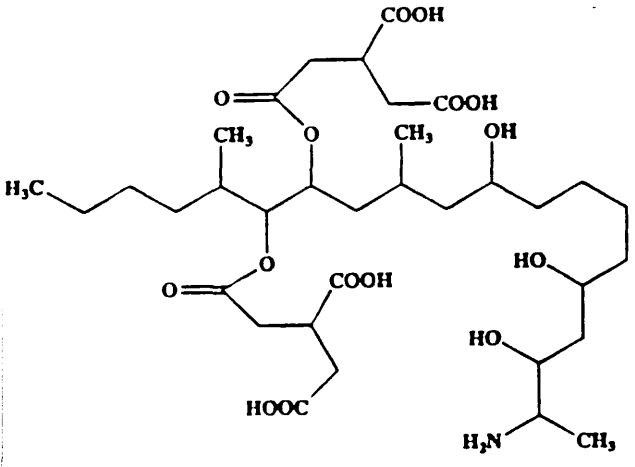
T-2 toxin

(a)



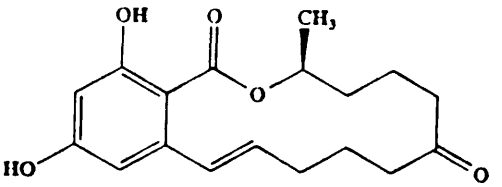
Deoxynivalenol

(b)



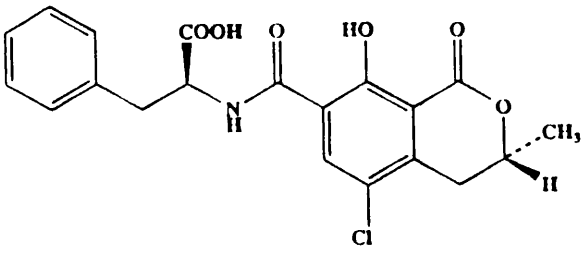
Fumonisin B_1

(c)



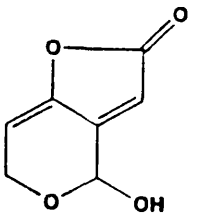
Zearalenone

(d)



Ochratoxin A

(e)



Patulin

(f)

Figure 2. The major mycotoxins produced by the *Fusarium* and *Penicillium* moulds.

acid and a pentahydroxyicosane containing a primary amino group. There is a marked similarity in chemical structure to sphingosine, a brain lipid. Fumonisins are highly toxic (Moss 1995b).

Fumonisin B_1 (Fig. 2c) is very water soluble, has considerable biological activity and is known to be responsible for equine encephalomalacia (Marasas *et al* 1988), porcine pulmonary oedema syndrome, hepatic cancer in the rat (Gelderblom *et al* 1992) and may be involved in the epidemiology of oesophageal carcinoma in humans in southern Africa and parts of China (Chu & Li 1994; Yoshizawa *et al.* 1994). Now that methods for its analysis have become more widely available, it appears to be quite common in maize and maize products (Sydenham *et al.* 1994).

Fusarin C is produced by at least nine species of *Fusarium* and seven of these species have been isolated from European agricultural crops and soils. Fusarin C has been showed to be highly mutagenic when tested on *Salmonella* TA 100 but less active than sterigmatocystin and aflatoxin B_1. This compound is potentially a carcinogen.

Moniliformin was originally isolated from *F. moniliforme* but has subsequently been isolated from at least 5 other species. This compound has been shown to be phytotoxic to wheat coleoptiles and to maize and tobacco plants. Moniliformin is extremely toxic to chicks and ducklings.

The most important producers of zearalenone (fig. 2d) are *Fusarium graminearum* and *F. semitectum*, while at least 8 other species can produce variable amounts of the toxin. In view of its low acute toxicity it has been suggested that a more appropriate definition would be a mycoestrogen. Zearalenone can be classified as a weak, non-steroidal, estrogen having anabolic properties.

The climatic factors that influence the establishment of *Fusarium* spp. in wheat and maize, the two crops of greatest concern from infection by these fungi, have been well researched. The fungi persist in the debris of plant tissue in the soil, where the ascospores and macroconidia form the resistant stage for survival. It is quite clear that

mycotoxin - producing strains of *Fusarium* are a world-wide problem. At one time it was considered that only temperature climates would allow the growth of the fungi but their isolation in India and Malaysia (Bhat *et al.* 1989) shows that this is not the case. A wide range of agricultural crops has been shown to be contaminated by *Fusarium* mycotoxins (Abramson *et al.* 1987). Wheat, maize, barley, oats, rye, sorghum, rice, potatoes, bananas, mustard seed, soyabeans, groundnuts, sunflower seed, cassava, have all been implicated.

The *Penicillium* Mycotoxins

The genus *Penicillium* contains many toxigenic species and historically because of the difficulty in accurately identifying these fungi there have been major errors in scientific papers concerned with *Penicillium* mycotoxins. However, taxonomic studies by Pitt (1989b) Samson (Samson & Pitt 1985), Frisvad (1986) and others are now bringing much greater authenticity to this field of mycotoxicology. The genus *Penicillium* and the derived mycotoxins are extremely complex and further exhibit great diversity. It is undoubtedly the most commonly occurring toxigenic group of fungi on foods and feedstuffs under normal storage conditions.

The main *Penicillium* mycotoxins found in the environment in significant amounts and to have some definite influence on human and animal health include cyclopiazonic acid, citrinin, ochratoxin A, patulin and penicillic acid. From those, only ochratoxin A (Fig. 2e) is subject to regulatory controls in some countries. Ochratoxin A (OTA) occurs widely on wheat, apparently causing porcine nephrosis and has been shown to induce tubular necrosis. The main producer species is now believed to be *Penicillium verrucosum. Penicillium* species are the primary sources of OTA in stored cereals in the temperature climates of Europe and North America, whereas *Aspergillus* strains are

commonly isolated in climates such as South Africa. OTA can contaminate all the major cereals, pulses and soya, coffee and cocoa beans.

Citrinin is the major yellow-rice toxin, produced by *Penicillium citrinin*. The OTA producing *P. verrucosum* is also a producer. Citrinin also occurs in cereals and in maize. It is a less potent toxin than OTA and LD_{50}s are known for mice, rats, rabbits and guinea pigs.

Cyclopiazonic acid, besides being produced by *Aspergillus flavus*, can also be formed by several penicillia including *P. commune* and *P. griseofulvum*. It is often produced along with aflatoxins but the dearth of human exposure data, mainly due to the lack of well-developed analytical methods for its determination in foods, precludes an assessment of possible health effects. It has been detected in naturally contaminated mixed feeds, turkey rations, maize, peanuts and other foods and feeds.

Patulin (Fig.2f) is produced principally by *Penicillium expansum*, the principal cause of apple rot and a common pathogen on many fruits and vegetables. It is a common contaminant of fruit juices, particularly apple juice but although the contamination incidence is quite high, the level of contamination is usually low at less than 100 ppb. If fruit juices are subjected to alcoholic fermentation, then more than 99% of the toxin is destroyed. Data on the carcinogenic potential are still incomplete but it does have adverse effects on the developing foetus in rats.

There are several reports of penicillic acid found in foods though it is produced by a wide variety of moulds, including *P. aurantiogriseum* and *P. simplicissimum* which themselves grow on foods. This would be due to the instability of the compound in which, as with patulin, its unsaturated lactone structure reacts readily with sulphydryl compounds and is rapidly converted to other products not found to be biologically active.

THE FUTURE

Aspergillus flavus species will continue to occupy a wide variety of human habitats. Despite our vast knowledge of *A. flavus* and of aflatoxin production , many questions needed to be answered before efficient control system can be established. More research is needed to understand the physiology, metabolism and nutritional requirements of aflatoxigenic fungi. Detection and identification of *A. flavus* species is another area that needs further development. The use of scanning electron microscopy, the development of selective media and the rise of immunoassay techniques seem to have promising potential in this regard.

The *F. graminearum* toxins, deoxynivalenol and zearalenone, in cereals can be eliminated by plant breeding (Snijders 1994). However, it will take one or two decades for commercial hybrids and cultivars to become widely used. Since the *F. moniliforme* toxin, fumonisin has just been discovered it is difficult to predict how long it will take to resolve this problem. There are fairly serious problems remaining even in describing the nature of the *F. moniliforme*-cereals association.

The evidence of mycotoxin involvement in foodborne human disease is getting stronger. Various studies around the world have shown the relationship between the occurrence of acute and chronic disease and ingestion of foods that are contaminated with mycotoxins. Thus efforts have to be made to prevent mold growth and mycotoxin production along the entire food chain, from field to table.

However, such efforts are almost impossible without the further research needed to answer numerous questions. First, there is need for total surveillance of foods and feeds for the presence of mycotoxigenic fungi and mycotoxins. Second, an efficient control system needs to be established. This could be accomplished through the development of efficient detoxification and decontamination procedures, worldwide regulations, and improvement of storage facilities, especially in developing countries. Third, there is

needed for better understanding of the toxicological effects of mycotoxins and fourth, sampling and analytical methods for mycotoxigenic fungi and mycotoxins must continue to be improved.

APPENDIX

SCIENCES DES ALIMENTS, 16(1996) 505-513

Sulphur dioxide binding and losses during processing and storage of dried figs

Maria A. MELANITOU [1], Dimitrios N. PAPANICOLAOU [1], William J. WHISH [2]

RÉSUMÉ Liaison et perte du dioxyde de soufre au cours du traitement et de la conservation de figues séchées.

Les facteurs influençant la perte du dioxyde de soufre, absorbé par les figues lors la fumigation et au cours du stockage ont été étudiés. Il a été constaté que le lavage à l'eau chaude des figues séchées appliqué avant l'emballage a pour conséquence la perte de 50 % de la teneur initiale en dioxyde de soufre du produit. Au cours du stockage des figues, les facteurs qui favorisent la rétention du dioxyde de soufre sont la température et la composition de l'atmosphère environnante. Il a été constaté que le stockage des figues sous vide a pour conséquence une perte du dioxyde de soufre plus rapide. La perte du dioxyde de soufre sous forme libre, combinée ou totale est plus importante à des températures de stockage élevées. Le dioxyde de soufre sous forme libre présente une perte plus rapide par rapport aux autres formes. Le matériel d'emballage joue également un rôle très important sur la rétention du dioxyde de soufre par les figues séchées. Une rétention satisfaisante du dioxyde de soufre a été obtenue après emballage du produit à l'aide d'un film laminé en polyéthylène-polypropylène haute densité.

Mots clés : Ficus carica, *fruit sulfité, conditionnement, atmosphère modifiée, séchage.*

SUMMARY

The factors influencing the loss of sulphur dioxide retained by dried figs after sulphur fumigation and during storage was studied. It was found that hot washing of figs before packaging halved the total sulphur dioxide content of dried figs. During subsequent storage, the temperature of storage and the composition of the surrounding atmosphere are the most important factors influencing the retention of sulphur dioxide by dried fig fruit. It was found that figs stored in packages under vacuum lose their sulphur dioxide most easily. The rate of sulphur dioxide loss, measured as either free, combined or total, increased with temperature. The most rapid loss was that of the free sulphur dioxide. Packaging material had also a consistent effect on sulphur dioxide retention by dried fig fruit. The use of a film from high density polyethylene-polypropylene laminate as packaging material was found to be satisfactory for preventing sulphur dioxide losses.

Key-words : Ficus carica, *sulphited fruit, packaging, modified atmosphere, drying.*

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1 - INTRODUCTION

Sulphur dioxide (SO_2) has long been used to retard the rate of browning and to maintain the natural colour in dried fruits such as apricots, peaches, pears and figs during sun-drying and storage. This preservative is absorbed by fruit tissue when it is exposed to the fumes from burning sulphur just before drying. Much of the absorbed sulphur dioxide is lost during drying and of that which remains, the greater part is in a combined form (MCBEAN, 1967). The retention of sulphur dioxide by fruits during sulphuring and drying has been reviewed by several authors (NICHOLS *et al.*, 1938; MCBEAN, 1967). This bound sulphite has no retarding effect on product deterioration. The sulphur dioxide retained by fruit is lost through storage either by physical loss or by conversion into combined sulphite or finally by oxidation to sulphate (GILBERT and MCWEENY, 1976; MCWEENY *et al.*, 1980).

First STADTMAN *et al.* (1946 a,b,c) in their studies of the factors which influence the rate of darkening of dried apricots during storage, showed the importance of sulphur dioxide level, moisture content, temperature and oxygen availability. Most of their experiments were done under carefully controlled laboratory conditions but at high temperatures (40-50°C). NURY *et al.* (1960) carried out similar experiments with dried apricots at storage temperatures ranging from 1.3 to 32.2°C. Both groups of investigators measured total sulphur dioxide but NURY *et al.* (1960), while showing that the rate of loss was higher in samples with higher initial levels, proposed that this may have been due to the presence of larger amounts of free sulphur dioxide. Package and environment were also found to affect the loss of sulphur dioxide during storage (DAVIES *et al.*, 1973, SAYAVEDRA and MONTGOMERY, 1983).

The figs of Kymi (Calimyrna variety with thin skin which permits a satisfactory penetration of sulphur dioxide) are usually sulphured in order to obtain a yellowish colour which is very desirable in the European market and which only occurs in this kind of fig. The purpose of our study was to investigate the changes in sulphur dioxide content (free and combined) during processing and storage of dried figs under various storage conditions in an attempt to provide information for the determination of shelf life of the product.

2 - MATERIALS AND METHODS

The figs used in the experiments were obtained from Kymi, Greece, sulphured by burning sulphur and sun-dried for 2-3 days, until a moisture content of 23-24% was reached. Before packaging, they were disinfected and then washed for 20 s with water at 60-70°C, dried again and finally packed. In order to study the effect of the packaging material on the loss of total sulphur dioxide content, three packa-

ging materials were used: the traditional paper box used for the retail market and two plastic films; one from low density polyethylene high barrier to water vapour and actual permeability to oxygen ca 35, carbon dioxide ca 90 and nitrogen ca 9 $ml.m^{-2}$ $day^{-1}bar^{-1}$ at 20°C (plastic A) and the other from a high density polyethylene-polypropylene laminate high barrier to water vapour and actual permeability to oxygen ca 15, carbon dioxide ca 35 and nitrogen ca 9 $ml.m^{-2}$ $day^{-1}bar^{-1}$ at 20°C (plastic B). In order to obtain the above permeabilities, the LPDE films were relatively thick (thickness of 430 µm). To study the effect of storage temperature on retention of sulphur dioxide, figs with the same initial total sulphur dioxide level were packed in plastic B – the bags were stored at 4°C, 25°C and 37°C in thermostatically controlled incubators. The packs were filled with figs (100 g), then evacuated, gas flushed and heat sealed by means of a HenKovac 1 700 automatic sealer equipped with a gas supply.

Figs with the same initial total sulphur dioxide level were packed in plastic B bags and stored at 25°C under three different atmospheric conditions: 100% air, 50% air-50% carbon dioxide and under vacuum. The concentration of gases was given by the company which supplied the canisters. The time of storage for all packs was 12 months. At frequent time intervals, packs were drawn and analyzed for total and free sulphur dioxide.

Total sulphur dioxide was determined by the Monier-Williams distillation method (SHIPTON, 1954). The samples were homogenized by grinding and 25 g portions were used to determine total sulphur dioxide content. Free sulphur dioxide was determined by the method developed by BURROUGHS and SPARKS (1964). Combined sulphur dioxide was calculated from the difference between free and total sulphur dioxide. Statistical analysis of variance was made with the STATGRAPHICS 6.0 software (Statistical Graphics Corporation, Portion Copyright 1992 Manugistics, Inc.).

3 - RESULTS AND DISCUSSION

3.1 Effect of hot-washing treatment

During the sulphur treatment, it is known that only a little of the absorbed sulphur dioxide is bound in the initial stage. During sun-drying, more of the sulphur dioxide becomes bound and some is totally lost from the system by vaporization and by oxidation into sulphate (MCBEAN, 1967). Our experiments showed that in dried figs, with an initial amount of total sulphur dioxide of 1.95 $g.kg^{-1}$, 83.6% is in bound form.

Hot-washing treatment before packaging seemed to drastically reduce the total sulphur dioxide retained by the fruit as the figs lose about 50% of their initial content (from 1.95 to 0.94 $g.kg^{-1}$). Contrary to the findings of MCBEAN *et al.* (1965), our experiments show a marked decrease in SO_2 retention after hot washing. This could be due to the fact that the figs were already dry, so there was little possibility of plasmolysis and sulphur dioxide absorption. On the other hand, hot-washing resulted in an increase in humidity of the product (from 18.5 to 21.7%).

3.2 Effect of packaging material

It is evident from the results of the present studie *(fig. 1)* that figs packed in highly permeable polyethylene have lost 60% of their initial total sulphur dioxide by the end of the storage period. However, figs in low gas permeable laminate have lost only 15% of their initial total sulphur dioxide during the same period *(fig. 1)*. On the other hand, figs in the paper packaging showed a loss similar to that of the highly permeable polyethylene. These results confirm the findings of DAVIES *et al.* (1973) for dried apricots. The dramatic loss of sulphur dioxide is followed by a loss of humidity which consequently results in a product of lower quality.

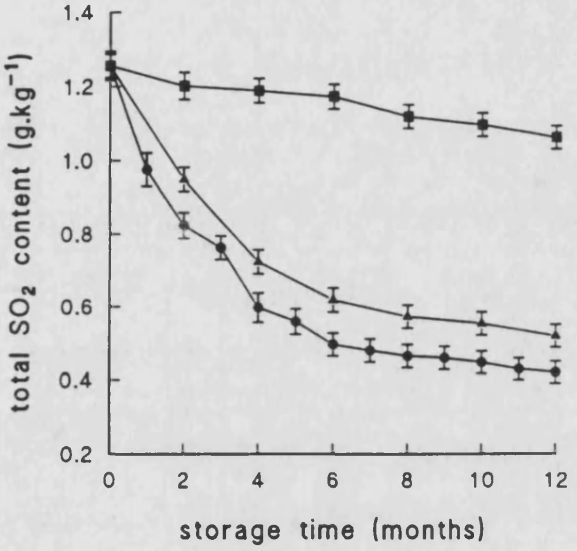

Figure 1

Effect of packaging material on loss of sulphur dioxide absorbed by dried figs packed under air and stored at 25°C
(• paper box; ■ polyethylene; ▲ polyethylene-polypropylene laminate)
Each point represents the average of three determinations

It seems that the plastic B package is a satisfactory alternative for preventing sulphur dioxide loss. Further studies should consider which part of sulphur dioxide is converted to sulphate in the plastic packaging. Presently this cannot be measured. It may be that the gas phase composition within the plastic bag changes due to diffusion of oxygen.

3.3 Effect of the composition of storage atmosphere

In the present experiment, the initial composition of the storage atmosphere seems also to affect the rate of loss of total sulphur dioxide. It is obvious that figs stored under vacuum lose their sulphur dioxide most easily, while CO_2-enriched atmosphere slowed the rate of loss of total sulphur dioxide *(fig. 2)*. On the other hand, the loss of total sulphur dioxide from fruit stored in air has, at the beginning, the same trend as with carbon dioxide storage but, after three months of storage,

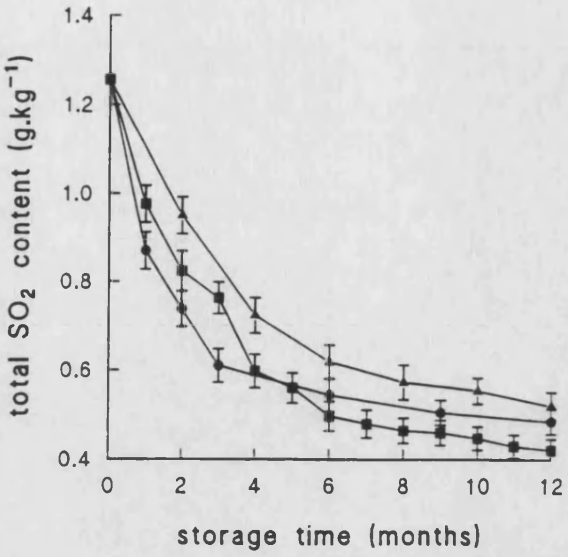

Figure 2

Effect of the composition of storage atmosphere on the loss of sulphur dioxide absorbed from dried figs at 25°C (■ 50% carbon dioxide-50% air; • Vacuum; ▲ 100% air)

Each point represents the average of five determinations

The package used is polyethylene-polypropylene bags under air

the trend "reaches" that of the vacuum storage. This may be due to the gradual loss of air through the package, which diminishes the available oxygen for reaction. As oxygen availability to the system is a major factor that influences sulphur dioxide binding (due to oxidation) the experiments done in the plastic B bags under three different atmospheres, suggest that carbon dioxide storage and to a lesser extent, air, are the most appropriate storage atmospheres for sulphured dried figs.

3.4 Effect of storage temperature

The storage temperature seems also to be an important factor in the loss of total sulphur dioxide. As can be seen in figure 3, cold storage (4°C) had a minor effect on the rate loss of sulphur dioxide, while higher temperatures (25°C or 37°C) hastened that loss. Sulphur dioxide measured as either total, combined or free *(fig. 3 and 4)* declined most rapidly at the highest storage temperature. Comparing these trends with each other, the most rapid loss occured with the free sulphur dioxide which resulted also in a concurrent loss in total sulphur dioxide, while bound sulphur dioxide loss was fairly constant. As more of the free sulphur dioxide was lost, the bound sulphur dioxide content became lower, because some of it was converted back to the free form *(fig. 5)*. These results are in agreement with the findings of STADTMAN *et al.* (1946a,b,c), DAVIES *et al.* (1973) in other dried fruits and BOLIN and JACKSON (1985).

From figure 5 it is also evident that storage temperature affects the binding of sulphur dioxide. Cold storage (4°C) prevented any significant binding but when the storage temperature was raised to 25°C, the degree of binding increased.

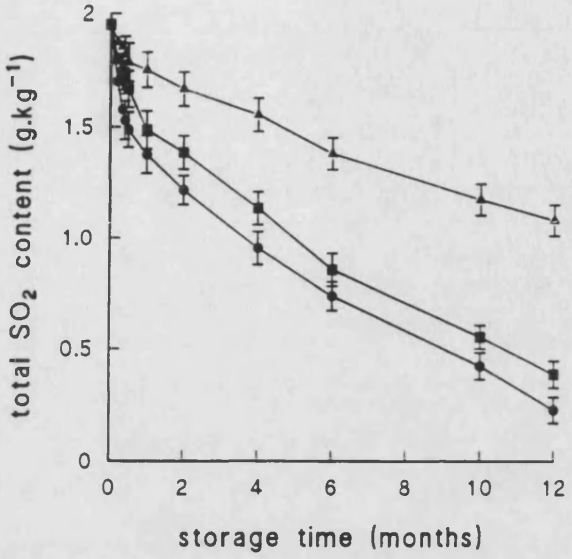

Figure 3

Effect of temperature of storage on loss of total sulphur dioxide absorbed from dried figs (▲ 4°C; • 25°C; ■ 37°C)

Each point represents the average of five determinations

The package used is polyethylene-polypropylene bags under air

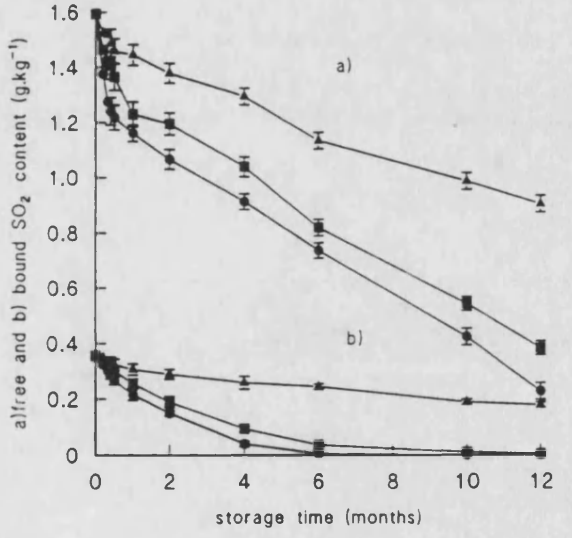

Figure 4

Effect of storage temperature on loss of a) free and b) combined sulphur dioxide absorbed from dried figs (▲ 4°C; • 25°C; ■ 37°C)

Each point represents the average of five determinations

The package used is polyethylene-polypropylene bags under air

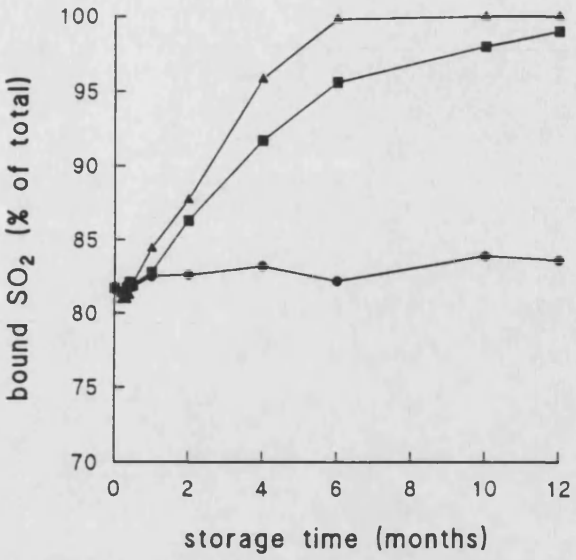

Figure 5

Effect of storage temperature on the degree of sulphur dioxide binding in dried figs (▲ 4°C;• 25°C;■ 37°C)

Each point represents the average of five determinations

The package used is polyethylene-polypropylene bags under air

Storage of the dried figs at 37°C resulted in a further increase of binding, possibly due to the activation energy of the binding reaction because of the higher temperature. From the above results, it can be concluded that an appropriate temperature for storage of dried figs would be around 20-25°C. Lower temperatures are more effective but not applicable, as on retail market shelves the temperatures are around 25°C. It is tempting to speculate from our findings that the initial amount of sulphur dioxide plays an important role on sulphur dioxide loss. A higher amount of initial sulphur dioxide is lost at a slower rate than a lower one *(fig. 2 and 3)*. Further studies need to be done, however, along with the identification of sulphur dioxide-binding compounds in figs, to establish the validity of this contention.

4 - CONCLUSIONS

The present study involved the rate of sulphur dioxide loss during storage of sulphured dried figs packed in cartons or plastic film. The processing treatments after sulphuring and before packaging lower the amount of sulphur dioxide retained by the figs to such a level as is permitted by Food Code regulations and also is enough to provide a safe storage. Among the parameters of storage, it is the temperature which has the most consistent effect on the loss of sulphur dioxide

from sulphited dried figs. It can be concluded, in combination with some other studies of the physicochemical and microbiological changes occurring during storage, that sulphited dried figs can be safely stored for 9-12 months under air in plastic bags made from a high density polyethylene-polypropylene laminate at 25°C.

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REFERENCES

BOLIN H.R., JACKSON R., 1985. Factors affecting sulfur dioxide binding in dried apples and apricots. *J. Food Process. Preserv.*, **9**, 25-34.

BURROUGHS L.F., SPARKS A.H., 1964. The determination of the free sulphur dioxide content of ciders, *The Analyst*, **89**, 55-60.

DAVIES E.G., McBEAN D., ROONEY M.L., GIBBS P.G., 1973. Mechanisms of sulfur dioxide loss from dried fruits in flexible films. *J. Food Technol.*, **8**, 391-405.

GILBERT J., McWEENY D.J., 1976. The reactions of sulphur dioxide in dehydrated vegetables. *J. Sci. Food Agric.*, **27**, 1145-1146.

McBEAN D.M., PITT J.I., JOHNSON A.A., 1965. Retention of absorbed sulfur dioxide in fruit tissue during drying. *Food Technol.*, **19**, 141-145.

McBEAN D.M., 1967. Levels of free and combined sulphur dioxide in fruits during sulphuring and drying. *Food Technol.*, **21**, 1402-1406.

McWEENY D.J., SHEPARD M.J., BATES M.L., 1980. Physical loss and chemical reactions of sulphur dioxide in strawberry jam production. *J. Food Technol.*, **15**, 613-617.

NICHOLS P.F., MRAK E.M., BETHEL R., 1938. Effect of drying and storage conditions on colour and sulfur dioxide retention of dried apricots. *Food Research*, **4**, 67-74.

NURY F.S., TAYLOR D.H., BREKKE J.E., 1960. Research for better quality in dried fruits. Figs. *U.S. Dept.Agric.* ARS-74-16, Washington D.C.

SAYAVEDRA L.A., MONTGOMERY M.W., 1983. Effect of moisture, temperature and sulfur dioxide on colour of dried apples. *In: Annual IFT Meeting 43rd Abstr.*, **301**.

SHIPTON J., 1954. Estimation of sulphur dioxide in dried foods. *Food Preserv. Quart.*, **14**, 54.

STADTMAN E.R, BARKER H.A., HAAS V., MRAK E.M., 1946a. Storage of dried fruit:

influence of temperature on deterioration of apricots. *Ind. Eng. Chem.*, **38**, 541-543.

STADTMAN E.R, BARKER H.A., HAAS V., MRAK E.M., MACKINNEY G., 1946b. Storage of dried fruit: gas changes during storage of dried apricots and influence of oxygen on rate of deterioration. *Ind. Eng. Chem.*, **38**, 324-329.

STADTMAN E.R, BARKER H.A., MRAK E.M., MACKINNEY G., 1946c. Storage of dried fruit: influence of moisture and sulphur dioxide on deterioration of apricots. *Ind. Eng. Chem.*, **38**, 99-104.

REFERENCES

Abdel-Mallek, A.Y., Hassan, H.A.H., Bagy, M.M.K. (1994). Efficacy of hydrocarbons against soybean seed-borne fungi and aflatoxin production. Microbiol. Res. 149(2), 173-180.

Abdulov, A. (1938). Preserving foods with sulfurous acid. Azerbaidzhan Med. Zhur. 2, 146-149.

Abramson, D., Clear, R.M., Nowicki, T.W. (1987). *Fusarium* species and trichothecene mycotoxins in suspect samples of 1985 Manitoba wheat. Can. J. Pl. Sci. 67, 611-619.

Adachi, T., Nonogi, H., Fuke, T., Ikuzawa, M., Fujita, K., Izumi, T., Hamano, T., Mitsuhashi, Y., Matsuki, Y., Suzuki, H., Toyoda, M. Ito, Y. and Iwaida, M. (1979). Studies on the analyses of sulphites in food. II. On the combination of sulphite with food ingredients (aldehydes, ketones and sugars). Z. Lebensm.-Untersuch. u. -Forschung 168(3), 200-205.

Akerstrand, K. & Moller, T. (1989). Examination of dried figs. Var Foda, 41, 308-317.

Altug, T. (1988). A comparison of Romer and CB methods for determination of aflatoxins in figs. Chem. Mikrobiol. Technol. Lebensm. 11(4), 97-99.

Altug, T., Yousef, A.E. and Marth, E.H. (1990). Degradation of aflatoxin B_1 in dried figs by sodium bisulfite with or without heat, ultraviolet energy or hydrogen peroxide. J. Food Prot. 53(7), 581-582.

Anderson, C., Warner, C.R., Daniels, D.H. and Padgett, K.L. (1986). Ion chromatographic determination of sulfites in foods. J. Assoc. Off. Anal. Chem. 69, 14-19.

Anonymous (1907). General results of the investigations showing the effect of sulphurous acid and sulphites upon digestion and health. U.S. Dept. Agr., Bur. Chem., Circ. No 37, 1-18.

Anonymous (1974). Imported figs recalled because of aflatoxin. Food Chem.News 16, 17-18.

Anonymous (1986). Mitt. Geb. Lebensmittelunters Hyg. 77, 400-401.

Arseculeratue, S.N., Desilva, L.M., Wijesundra, S. and Bandunatha, C.H.S.R. (1969). Coconut as a medium for the experimental production of aflatoxin. J. Appl. Microbiol., 18, 88.

Ashworth, L.J.,Jr, McMeans, J.L. and Brown, C.M. (1968). Infection of cotton by *Aspergillus flavus*: the influence of temperature and aeration. Phytopathology, 59, 383-385.

Askin, O. & Kösker, O. (1980). Kuru incirler de aflatoxin olusumu uzerine bir arastirma. Ankara Universitesi, Ziraat Fakultesi Ihtisas Tez Ozetleri 1, 226-241. (Turkey).

Association of Official Analytical Chemists (1984a). Sulphur dioxide and total sulphurous acid in dried fruit. Official methods of Analysis, 14th edn. Association of Official Analytical Chemists, Arlington, VA, 10.085-10.088, 20.123-20.125.

Association of Official Analytical Chemists (1984b). Invert sugar in sugars and sirups; Lane-Eynon general volumetric method. Official methods of Analysis, 14th edn. Association of Official Analytical Chemists, Arlington, VA, 31.034-31.036.

Association of Official Analytical Chemists (1984c). Aflatoxins in peanuts and Peanut products. Official methods of Analysis, 14th edn. Association of Official Analytical Chemists, Arlington, VA, 26.026-26.031.

Atawodi, S.E, Atiku, A.A. & Lamorde, A.G. (1994). Aflatoxin contamination of Nigerian foods and feedingstuffs. Food and Chem. Toxicol. 32(1), 61-63.

Audu, T.O.K., Loncin, M. and Weisser, H. (1978). Sorption isotherms of sugars. Lebensmittel Wissenschaft und Technologie 11, 31-34.

Azer, M. & Cooper, C. (1991). Determination of aflatoxins in foods using HPLC and a commercial ELISA system. J.Food Prot. 54(4), 291-294.

Badii, F., Moss, M.O., Wilson, K. (1986). The effect of sodium biselenite on the growth and aflatoxin production of *Aspergillus parasiticus* and the growth of aspergilli. Letters in Applied Microbiology 2, 61-64.

Badii, F. & Moss, M.O. (1988). The effect of the fungicides tridemorph, fenpropimorph and fenarimol on growth and aflatoxin production by *Aspergillus parasiticus* Speare. Letters in Applied Microbiology 7, 37-39.

Baker, E.E. & Mrak, E.M. (1938). Yeasts associated with the "sugaring" of dried prunes and figs. J.Bacteriol. 36, 317-318.

Baker, R.C, Qureshi, R.A. and Hotchkiss, J.H. (1986). Effects of an elevated level of CO_2-containing atmosphere on the growth of spoilage and pathogenic bacteria at 2°C, 7°C and 13°C. Poultry Sci. 65, 729-737.

Banks, J.G. & Board, R.G. (1981). Sulfite- the elective agent for the microbial associations in British fresh sausages. J. Appl. Bact. 51, ix.

Banks, J.G. & Board, R.G. (1982). Comparison of methods for the determination of free and bound sulphur dioxide in stored British fresh sausage. J.Sci.Food Agric., 33, 197-203.

Barger, W.R. (1941). Effect of cold storage conditions on the keeping of dried fruits. Western Canner and Packer 33(6), 47-50.

Barger, W.R., Pentzer, W.T. and Fisher, C.R. (1948). Low temperature storage retains quality of dried fruit. Food Indus. 20, 337-340.

Baur, F.J. (1974). Effect of storage upon aflatoxin levels in peanut materials. J. Am. Oil Chem. Soc. 52, 263.

Beavens, E. A. & Bourne, J.A. (1945). Commercial sulfiting practices. Food Inds. 17(9), 1044-1045.

Beebe, R.M. (1978). Reverse phase high pressure liquid chromatographic determination of aflatoxins in foods. J.Assoc.Off.Anal.Chem. 61(6), 1347-1352.

Bennett, J.W., Fernholz, F.A. and Lee, L.S. (1978). Effect of light on aflatoxins, anthraquinones and sclerotia in *Aspergillus flavus* and *Aspergillus parasiticus*. Mycologia 70, 104-116.

Beuchat, L.R. (1987). Food and beverage mycology, 2nd ed., Van Nostrand Reinhold, New York, 28, 83-102.

Beutler, H.O. (1984). A new enzymatic method for determination of sulphite in food. Food Chem. 15, 157-164.

Beythien, A. & Bohrisch, P. (1902). Uber amerikanisches, getrocknetes Obst. Zeits. Untersuch.Nahr. und Genussm. 5, 401-409.

Bhat, R.V., Beedu, S.R., Ramakrishna, Y., Munshi, K.L. (1989). Outbreak of trichothecene mycotoxicosis associated with consumption of mould-damaged wheat in Kashmir valley, India. Lancet 7 jan, 35-37.

Bioletti, F.T. (1911). Sulfurous acid in wine making. Orig.Com. 8th Intern.Congr.Appl.Chem. 14, 31-59.

Bioletti, F.T. & Cruess, W.V. (1912). Enological investigations. Calif. Agr.Expt.Stat.Bull. 230, 25-64.

Bolin, H.R. (1980). Relation of moisture to water activity in prunes and raisins. J. Food Sci. 45(5), 1190-1192.

Bolin, H.R. & King, A.D. (1980). Figs. In "Tropical and subtropical fruits: composition, properties and uses" eds. S. Nagy, P.E. Shaw, pp. 492-505. AVI Publishing Westport Conn.

Bolin, H.R. & Jackson, R. (1985). Factors affecting sulfur dioxide binding in dried apples and apricots. J. Food Process. Preserv. 9(1), 25-34.

Bone, D.P. (1969). Water activity-its chemistry and applications. Food Prod. Dev. 3(5), 81-94.

Boyacioglü, D. & Gönül, M. (1990). Survey of aflatoxin contamination of dried figs grown in Turkey in 1986. Food Additives and Contaminants 7(2), 235-237.

Brekke, J.E. (1963). Collaborative study on assay of sulfur dioxide in dried apricots. J. Assoc. Off. Anal. Chem. 46(4), 618-622.

Buchanan, J.R., Sommer, N.F. and Fortlage, R.J. (1975). *Aspergillus flavus* infection and aflatoxin production in fig fruits. Appl. Microbiol. 30, 238-41.

Buchanan, J.R., Sommer, N.F. and Fortlage, R.J. (1985). Aflatoxin suppression by Modified atmospheres containing carbon monoxide. J.Am.Soc. Hort. Sci. 110(5), 638-641.

Buchi, G. & Rae, I.D. (1969). The structure and chemistry of aflatoxins. In "Aflatoxins". Ed. L.A. Goldblatt. Academic Press, New York, 55.

Bullerman, L.B. (1979). Significance of mycotoxins to food safety and human health. J.Food Prot. 42(1), 65-86.

Bullock, E., Roberts, J.C., and Underwood, J.G. (1962). Studies in mycological chemistry. XI. The structure of isosterigmatocystin and an amended structure of sterigmatocystin. III, J. Chem. Soc. 4179.

Burroughs, L.F. & Sparks, A.H. (1964). The identification of sulphur dioxide-binding compounds in apple juices and ciders. J. Sci. Food Agric. 15, 176-185.

Burroughs, L.F. & Sparks, A.H. (1973a). Sulphite-binding power of wines and ciders. II. Theoretical considerations and calculation of sulphite-binding equilibria. J.Sci. Food Agric. 24, 199-206.

Burroughs, L.F. & Sparks, A.H. (1973b). Sulphite-binding power of wines and ciders. III. Determination of carbonyl compounds in a wine and calculation of its sulphite-binding power. J.Sci. Food Agric. 24, 207-217.

Burton, H.S., McWeeny, D.J and Biltcliffe, D.O. (1963). Non enzymic browning: the role of unsaturated carbonyl compounds as intermediates and of sulfur dioxide as inhibitor of browning. J. Sci. Food Agric. 14, 911-920.

Bushell, M.E. (1989). The process physiology of secondary metabolite production. In *Microbial Products: New Approaches* (ed. S. Baumberg, I.S. Hunter & P.M. Rhodes) pp. 95-120. Cambridge University Press: Cambridge. U.K.

Caldis, P.D. (1927). Etiology and transmission endosepsis (internal rot) of the fruit of the fig. Hilgardia 2, 287-328.

Caldis, P.D. (1930). Souring of figs by yeasts and the transmission of the disease by insects. J.Agr.Res. 40(11), 1031-1051.

Carr, J.G., Davies, P.A. and Sparks, A.H. (1976). The toxicity of sulphur dioxide toward certain lactic acid bacteria from fermented apple juice. J. Appl. Bacteriol. 40, 201-212.

Chace, E.M., Church, C.G. and Sorber, D.G. (1930). Large scale experiments in sulfuring apricots. I. ind. Eng. Chem. 22, 1317-1320.

Chace, E.M., Church, C.G. and Sorber, D.G. (1933). Large scale experiments in sulfuring apricots. II. Effect of dehydrating, shade, drying and blanching. Ind. Eng. Chem. 25, 1366-1370.

Chinachoti, P. & Steinberg, M.P. (1984). Interaction of sucrose with starch during dehydration as shown by water sorption. J. Food Sci. 49, 1604-1608.

Choudary, A.K. & Sinha, K.K. (1993). Competition between a toxigenic *Aspergillus flavus* strain and other fungi on stored maize kernels. J. Stored Prod. Res. 29(1), 75-80.

Chu, F.S., Li, G.Y. (1994). Simultaneous occurrence of fumonisin B_1 and other mycotoxins in mold corn collected from the People's Republic of China in regions with high incidences of oesophageal cancer. Appl. Envir. Microbiol. 60, 847-852.

Claypool, L.L. & Ozbek, S. (1952). Some influences of temperature and CO_2 on the respiration and storage life of the mission figs. Proc. Am. Soc. Hort. Sci. 60, 226-230.

Condit, I.J. (1932). The structure and development of flowers of *Ficus carica.* Hilgardia 6, 443-481.

Condit, I.J. (1933). Fig culture in California. Calif.Agr.Expt.Stat.Circ. 77, 1-67.

Condit, I.J. (1947). The fig. Chronica Botanica Co. Waltham Mass.

Condit, I.J. (1969). Ficus: the exotic species. Berkeley California.

Copley, M.J. & Van Arsdel, W.B. (1964). Food Dehydration. Vol. II. p. 540.

Cruess, W.V. (1912). The effect of sulfurous acid on fermentation organisms. J. Ind. Eng. Chem. 4, 581-585.

Cruess, W.V., Richert, P.H. and Irish, J.H. (1931). The effect of pH on the toxicity of several preservatives to microorganisms. Hilgardia 6(10), 295-314.

Cruess, W.V. (1948). Commercial fruit and vegetable products. McGraw-Hill Book Company, New York, 3rd ed.

Cvetnic, Z. (1994). Cyclopiazonic acid and aflatoxin production by cultures of *Aspergillus flavus* isolated from dried beans and maize. Nahrung 38(1), 2125.

Davey, A.E. & Smith, R.E. (1933). The epidemiology of fig spoilage. Hilgardia 7, 523-551.

Davies, N.D & Diener, U.L. (1968). Growth and aflatoxin production by *Aspergillus parasiticus* by various carbon sources. Appl. Microbiol., 16, 158.

Davies, E.G., McBean, D., Rooney, M.L. and Gibbs, P.G. (1973). Mechanisms of sulfur dioxide loss from dried fruits in flexible films. J. Food Technol. 8, 391-405.

Davis, N.D. & Diener, U.L. (1980). Confirmatory test for the high pressure liquid chromatographic determination of aflatoxin B_1. J.Assoc. Off. Anal. Chem. 63(1), 107-109.

Davis, N.D., Barnett, D. and Moy, P.M. (1983). Determination of molecular and free sulphur dioxide in foods by headspace gas chromatography. J. Food Technol. 18, 233-240.

Detroy, R.W., Lillehoj, E.B. and Ciegler, A. (1971). Aflatoxins and related compounds. In "Microbial toxins" Vol.6, Eds.A. Ciegler,S. Kadis & S.J. Ajl, Academic Press, New York, 39.

Diener, U.L., Cole, R.J., Sanders, T.H., Payne, G.A., Lee, L.J., Klich, M.A. (1987). Epidemiology of aflatoxin formation by *Aspergillus flavus.* Ann. Rev. Phytopath. 25, 249-270.

Diener, U.L. & Davis, N.D. (1967). Limiting temperature and relative humidity for growth and production of aflatoxin and free fatty acids by *Aspergillus flavus* in sterile peanuts. J.Am. Oil Chem. Soc. 44, 259-263.

Diener, U.L. & Davis, N.D. (1969). Aflatoxin formation by *Aspergillus flavus.* in "Aflatoxins" ed. L.A. Goldblatt, Academic Press, New York, 13-54.

Diener, U.L. & Davis, N.D. (1970). Limiting temperature and relative humidity for aflatoxin production by *Aspergillus flavus* in stored peanuts. J. Am. Oil Chem. Soc. 47, 347.

Diew, S.W. & Demain, A.L. (1977). Effects of primary metabolism on secondary metabolism. Ann. Rev. Microbiol. 31, 343.

Dixon, R.C. & Hamilton, P.B. (1981). Effects of feed ingredients on the antifungal activity of propionate. Poultry Sci. 60, 2407.

Dorner, J.W. & Cole, R.J. (1989). Comparison of two ELISA screening tests with liquid chromatography for determination of aflatoxins in raw peanuts. J.Assoc. Off. Anal. Chem. 72(6), 962-964.

Doster, M.A. & Michailides, T.J. (1994). *Aspergillus* Molds and aflatoxins in pistachio nuts in California. Phytopathology 84, 583-590.

Doster, M.A., Michailides, T.J., Morgan, D.P. (1996). *Aspergillus* species and mycotoxins in figs from California orchards. Plant Disease 80(5), 484-489.

Dott, W. & Truper, H.G. (1978). Do sulphite-forming yeasts have a growth advantage linked with a killer property? Wein Wissenschaft 33(2), 143-145.

Doyle, M.P., Applebaum, R.S., Brackett, R.E. and Marth, E.H. (1982). Physical, chemical and biological degradation of mycotoxins in foods and agricultural commodities. J. Food Prot. 45, 964.

Doyle, M.P. & Marth, E.H. (1978a). Bisulfite degrades aflatoxin: effect of temperature and concentration of of bisulfite. J. Food Prot. 41, 774-780.

Doyle, M.P. & Marth, E.H. (1978b). Bisulfite degrades aflatoxin: effect of citric acid and methanol and possible mechanisms of degradation. J. Food Prot. 417, 891-896.

Dupuy, P. & Mangenet, J. (1963). Conditions du développment des bactéries acétiques et mésures préventines dans la conservation des vins. Ann. Technol. Agric. 12, 63.

Dyett, E.J. & Shelley, D. (1966). The effects of sulphite as preservative in British fresh sausages. J. Appl. Bact. 29, 439-446.

Eheart, J.F. & Mason, B.S. (1967). Sugar and acid in the edible portion of fruits. J. Am. Diet. Assoc. 50, 130.

Eichner, K. & Karel, M. (1972). The influence of water content and water activity on the sugar amino-browning reaction in model systems under various conditions. J. Agric. Food Chem. 20, 218.

Eichner, K. (1986). The influence of water content and water activity on chemical changes in foods of low moisture content under packaging aspects. In "Food Packaging and Preservation" ed. M. Mathlouth. Elsevier Applied Science Publishers, London pp. 67-92.

Eisen, G. (1901). The fig. U.S. Dept. Agr. Div. Pom. Bull. 9, 1-317.

Everett, T.H. (1981). *Ficus*-fig. The New York Botanical Garden Illustraded Encyclopedia of Horticulture. Vol.4 Garland Publishing Inc., New York, 1361-1371.

Finoli, C., Galli, A., Vecchio A., Locatelli, D.P. (1994). Hygienic aspects of fruit confectioneries. Industrie Alimentari 33(332), 1201-1206.

Fisher, C.D., Mrak, E.M. and Long, J.D. (1942). Effect of time and temperature of sulfuring on absorption and retention of sulfur dioxide by fruits. Fruit Prod. Jour. and Amer. Vinegar Indus. 21, 175-176, 199-200, 217, 219, 237-238.

Food and Agriculture Organization of the United Nations, Rome (1979). FAO, Food Nutrition Paper 13. Selected documents of the Joint FAO/WHO/UNEP Conference on mycotoxins, Nairobi, September 19-21, 1977.

Food Science Laboratory of Ministry of Agriculture Fisheries and Food (1989). Aflatoxins in figs. Sampling and experimental method. Norwitch September 1989.

Frazier, W.C. (1967). Food Microbiology. McGraw-Hill Company. New York and London.

Frisvad, J.C. (1986). Taxonomic approaches to mycotoxin identification (taxonomic indication of mycotoxin content of food). In Modern Methods in the Analysis and Structural Elucidation of Mycotoxins ed. Cole, R.J. pp.415-457, Orlando, Florida:Academic Press.

Gehman, H. & Osman, E.M. (1954). The chemistry of the sugar-sulfite reactions and its relationship to food problems. Advances in Food Research 5, 53-96.

Gelosa, L. (1990). Aflatoxin researsh in imported dried figs. Industrie Alimentari 29(278), 25-27.

Genesis 19, 24.

Gerderblom, W.C.A., Jackiewicz, K., Marasas, W.F.O., Thiel, P.G., Horak, R.M., Vleggar, R., Kriek, N.P.J. (1988). Fumonisins-novel mycotoxins with cancer-promoting activity produced by *Fusarium moniliforme*. Appl. Envir. Microbiol. 54, 1806-1811.

Gerderblom, W.C.A., Semple, E., Marasas, W.F.O., Farber, E. (1992). The cancer-initiating potential of the fumonisin B mycotoxins. Carcinogenesis 13, 433-437.

Gilbert, J. & McWeeny, D.J. (1976). The reactions of sulphur dioxide in dehydrated vegetables. J.Sci. Food Agric. 27, 1145-6.

Gilbert, J. (1989). Surveillance and control of aflatoxin examination of dried figs and fig paste imported into the U.K. International Symposium on Dried Figs and Aflatoxins, Izmir, Turkey, 4-8 April 1989.

Goldblatt, L.A. (1972). Implications of mycotoxins. Clin. Toxicol., 5, 453.

Golumbic, C. (1965). Fungal spoilage in stored crops. In "Mycotoxins in Foodstuffs" G.N. Wogan Ed. MIT Press, Cambridge Massachusetts.

Golumbic, C. & Kulik, M.M. (1969). Fungal spoilage in food crops and its control. In "Aflatoxins" L.A.Goldblatt Ed. Academic Press NY, 311.

Gouda, M.S. Zoueil, M.B. El-Zalaki, E.M. and Mohamed, M. (1975). Technological studies on fig varieties planted in Arab Republic of Egypt. Alexandria J. Agr. Research 23(3), 459-466.

Gould, G.W. & Russell, N.J. (1991). Sulphite. In "Food preservatives" eds. N.J. Russell, G.W. Gould Blackie and Son Ltd, Glasgow and London, 72-87.

Gourama, H. & Bullerman, L.B. (1995a). Antimycotic and antiaflatoxigenic effect of lactic acid bacteria:a review. J. Food Prot. 57(11), 1275-1280.

Gourama, H. & Bullerman, L.B. (1995b). Inhibition of growth and aflatoxin production of *Aspergillus flavus* by *Lactobacillus* species. J. Food Prot. 58(11), 1249-1256.

Greek Agricultural Statistic Annual Review (1981)

Greek Food Code (1984). Periodikos Typos, Athens p.284.

Green, L.F. (1976). Sulfur dioxide and food preservation- a review. Food Chem. 1, 103-124.

Gregory, J.F. & Manley, D. (1981). High performance liquid chromatographic determination of aflatoxins in animal tissues and products. J. Assoc. Off. Anal. Chem. 64(1), 144-151.

Grncarevic, M. & Lewis, W. (1973). External colour of dried sultanas. Food Techn. in Australia 25(11), 562-565.

Hagler, W.M.,Jr, Hutchins, J.E. and Hamilton, P.B. (1982). Destruction of aflatoxin B_1 in corn with some sodium bisulfite. J.Food Prot. 45, 1287-1291.

Hagler, W.M., Hutchins, J.E. and Hamilton, P.B. (1983). Destruction of aflatoxin B_1 with sodium bisulfite: isolation of the major product aflatoxin B_1S. J. Food Prot. 46, 295-300.

Haisman, D.R. (1974). The effect of sulphur dioxide on oxidizing enzyme systems in plant tissues. J.Sci. Food Agric. 24, 207-217.

Hamman, V. (1951). Die Verwendung chemischer Konservierungsmittel in Lebensmittel-verkehr. Z. Lebensm.-Untersuch. und Forschung 93, 93-101.

Hammond, S.M. & Carr, J.G. (1976). The antimicrobial activity of SO_2 with particular reference to fermented and non-fermented fruit juices. In "Inhibition and inactivation of vegetative microbes" eds F.A.Skinner, W.B. Hugo, p:89-110, Academic Press.

Hansen, H.N. & Davey, A.E. (1932). Transmission of smut and mold in figs. Phytopathology 22, 247-252.

Hara, S., Fennell, D.L. and Hesseltine, C.W. (1974). Aflatoxin-producing strains of *Aspergillus flavus* detected by fluorescence of agar medium under U.V. light. Appl. Microbiol. 27, 1118-1123.

Harwig, J., Chen, Y-K., Kennedy, B.P.C., and Scott, P.M. (1973). Occurrence of patulin and patulin producing strains of *Penicillium expansum* in natural rots of apple in Canada. Can. Inst. Food Sci. Technol. J. 6, 22.

Hegazi, S.M. & Salem, S.A. (1977). Carbohydrates and amino acids of Egyptian figs. Sudan Journal of Food Science and Technology 9, 68-70.

Hendel, C.E., Bailey, G.F. and Taylor, D.H. (1950). Measurement of non-enzymatic browning of dehydrated vegetables during storage. Food Technol. 4, 344-347.

Hesseltine, C.W., Shotwell, O.L., Ellis, J.J. and Stubblefield, R.D. (1966). Aflatoxin formation by *Aspergillus flavus*. Bacteriol. Rev. 30, 795-805.

Hesseltine, C.W., Shotwell, O.L., Smith, M., Ellis, J.J., Vandergraft, E. and Shannon, G. (1985). Production of various aflatoxin strains of the *Aspergillus flavus* series, Proc. First U.S. Jpn. Conf. Toxicol. Microorg. 1970, in "Trichothecenes and other mycotoxins", ed. J. Lacey, John Wiley & Sons, Chichester, 258.

Hocking, A.D. & Pitt, J.I.(1986). A selective medium for rapid detection of Aspergillus flavus. In "Methods for the mycological examination of food". A.D.Jr King, J.I. Pitt, L.R. Beuchat and J.E.L. Corry Eds. NATO ASI Series A, Vol. 22, 127-132.

Hodge, J.E. (1953). Dehydrated foods. Chemistry of browning reaction in model systems. J.Agric. Food Chem. 1, 928-943.

Holmquist, G.U., Walker, H.W and Stahr, H.M. (1983). Influence of temperature, pH, water activity and antifungal agents on growth of *Aspergillus flavus* and *Aspergillus parasiticus*. J. Food Sci. 43, 778-782.

Holzapfel, C.W., Purchase, I.F.H., Steyn, P.S. and Gouws, L. (1966). The toxicity and chemical assay of sterigmatocystin, a carcinogenic mycotoxin and its isolation from two new fungal sources. S. Afr. Med. J., 40, 1000.

Howard, L.O.C. (1901). Smyrna fig culture in the United States. U.S. Dept. Agr. Yearbook 1900, 79-106.

Hunt, D.C., Bourdon, A.T., Wild, P.J. and Crosby, N.T. (1978). Use of High Performance Liquid Chromatography combined with fluorescence detection for the identification and estimation of aflatoxins and ochratoxin in food. J.Sci. Food Agric. 29, 234-238.

Hussain, M. & Vojir, F. (1993). A sampling plan for the control of aflatoxin B_1 in imported dried figs. Deutsche Lebens. Rund. 89 (12), 379-383.

Hutchinson, R.D., & Holzapfel, C.W. (1971). The conversion of sterigmatocystin into dihydroaspertoxin. Tetrahedron Lett. 27, 425.

Ingles, D.L. (1966). Prevention of non-enzymatic browning. CSIRO Food Pres. Quarterly, 26, (2,3,4).

Ingram, M. (1948). Germicidal effects of free and combined sulphur dioxide. J. Soc. Chem. Ind. 67, 18-21.

Ingram, M. (1949). Behavior of sulphur dioxide in concentrated orange juice. Food Research 14, 54-71.

Jackson, D. (1986). Figs. In "Temperate and subtropical fruit production" Butterworths of New Zealand Ltd.

Jennings, W.Q. (1977). Volatile components of figs. Food Chem. 2(3), 185-191.

Jewell, W.R. (1927). Sulfuring dried fruit. J. Dept. Agr. Victoria. 25, 457-462.

Joffe, A.Z. & Lisker, N. (1969). Effect of light, temperature and pH value on aflatoxin production in vitro. Appl. Microbiol. 18, 517.

Joslyn, M.A. & Braverman, J.B.S. (1954). The chemistry and technology of the pre-treatment and preservation of fruit and vegetable products with sulfur dioxide and sulfites. Adv.Fd.Res. 97-160.

Joslyn, M.A & Mrak, E.M. (1933). Investigations on the use of sulfurous acid and sulfites in the preparation of fresh and frozen fruit for baker's use. Fruit Products J. 12, 135-140.

Joslyn, M.A & Ponting, J.D. (1951). Enzyme-catalyzed oxidative browning of fruit products. Advances in Food Research 3, 1-44.

Kalogereas, S.A. (1932). Studies and experiments on fig sterilization. Greek National Press.

Kalogereas, S.A. (1935). An approach in the study of fig technology in Greece. Phd Thesis, Univ. of Thessaloniki, Faculty of Chemistry Greece.

Kim, K.H. (1981). Chemical components of korean fig and its storage stability. Korean J. Food Sci. Technol. 13(2), 165-169.

King, A.D.Jr., Fields, R.K. and Boyle, R.P. (1968). Dried fruits have low microbial counts. Food Engin. 40(12), 82-83.

Kocabas, N. & Karapinar, M. (1993). Evaluation of plating media and pouring temperatures for enumeration of fungi in dried figs. Doga Turkish Journal of Agriculture and Forestry 17(1), 205-211.

Komanowski, M., Talley, F.B. and Eskew, R.K. (1970). Air drying of cultivated mushrooms. Food Technol. 24, 80-84.

Kuehn, H.H., & Gunderson, M.F. (1963). Psychrophilic and mesophilic fungi in frozen food products. Appl. Microbiol. 11, 352.

Kurtzman, C.P., Horn, B.W. and Hesseltine, C.W. (1987). Antonie van Leeuwenhoek, J.Microbiol.Serol. 53, 147.

Labuza, T.P. & Saltmarch, M. (1978). The non-enzymatic browning reaction as affected by water in foods. In "Water activity: influences on food quality" Eds L.B. Rockland, G.F. Stewart, Academic Press. New York, London.

Labuza, T.P. (1982). Moisture gain and loss in packaged foods. Food Techn. 36(4), 92-97.

Lancaster, M.C., Jenkins, F.P. and Phillip, J.M. (1961). Toxicity associated with certain samples of groundnuts. Nature 192, 1095-1096.

Landers, K.E., Davis, N.D. and Diener, U.L. (1967). Influence of atmospheric gases on aflatoxin production by *Aspergillus flavus* in peanuts. Phytopathology, 57, 1086.

Legault, R.R., Hendel, C.E., Talburt, W.F. and Rasmussen, L.B. (1949). Sulfite disappearance in dehydrated vegetables during storage. Ind. Eng. Chem. 41, 1447-1451.

Legault, R.R., Hendel, C.E., Talburt, W.F. and Pool, M.F. (1951). Browning of dehydrated sulfited vegetables during storage. Food Techn. 5(10), 417-423.

Lenovich, L.M., Walters, J.L. and Reed, D.M. (1986). Comparison of media for the enumeration of fungi from dried foods. In "Methods for the mycological examination of food" Eds. A.D.Jr King, J.I. Pitt, L.R. Beuchat and J.E.L. Corry NATO ASI Series A, Vol.22, 76-83.

Lie, Jennie L. & Marth, E.H. (1968). Aflatoxin formation by *Aspergillus flavus* and *Aspergillus parasiticus* in a casein substrate at different pH values. J. Dairy Sci. 51, 1743.

Lin, M.T. & Dianese, J.C. (1976). A coconut-Agar medium for rapid detection of aflatoxin production by Aspergillus species. Phytopathology 66, 1466-1469.

Llewellyn, G.C. & Eadie, T. (1974). Evaluating the potential for occurrence of aflatoxins in black sunflower seeds. J.Assoc. Off. Anal. Chem. 57, 858-860.

Lockhead, A.G & Farrell, L. (1936). Effect of preservatives on fermentation and viability of sugar-tolerant yeasts. Food Research 1, 517-524.

Long, J.D., Mrak, E.M. and Fischer, C.D. (1940). Investigations in the sulfuring of fruits for drying. Bull. 635, Univ. of California.

Lötter, L.H. & Kröhm, H.J. (1988). Occurrence of aflatoxin in human foodstuffs in South Africa. Bull. Environ. Contam. Toxicol. 40, 240-243.

Lueck, E. (1980). Antimicrobial Food Additives. Springer-Verlag, Berlin, Heidelberg.

M.A.F.F. (1993). Mycotoxins:Third Report Food Surveillance Paper No 36. HMSO :London.

M.A.F.F., (1994). Aflatoxins in herbs and spices. Food Safety Information Bulletin No. 45, 3-4.

Makower, B. & Dehority, G.L. (1943). Equilibrium moisture content of dehydrated vegetables. Ind. Eng. Chem. 35, 193-197.

Mangan, J.L. & Doak, B.W. (1949). Behavior of sulfur dioxide in dehydrated vegetables. New Zealand J. Sci. Technol. 30(4), 232-236.

Marakis, S. (1990). Principles of Applied Microbiology. Univ. of Athens. School of Biol. Sci. Greece.

Marasas, W.F.O., Kellerman, T.S., Gelderblom, W.C.A., Coetzer, J.A.W., Thiel, P.G., van der Lugt, J.J. (1988). Leucoencephalomalacia in a horse induced by fumonisin B_1 isolated from *Fusarium moniliforme.* Onderspoort J. Vet. Res. 55, 197-203.

Marsh, P.B., Simpson, M.E., Ferretti, R.J., Campbell, T.C. and Donoso, J. (1969). Relation of aflatoxins in cotton seeds at harvest to fluorescence in the fiber. J. Agr. Food Chem. 17(3), 462-467.

Masson, A. & Meier, P. (1988). Contamination of spices, dried or frozen mushrooms and Turkish figs by moulds. Microbiologie-Aliments-Nutrition 6, 403-406.

Mateles, R.I. & Adye, J.C. (1965). Production of aflatoxin in submerged cultures. Appl. Microbiol. 13, 208-211.

Mason, H.S. (1953). The structure of melanins. In "Pigment cell growth", Proceedings of the third Conference on the biology of normal and atypical pigment cell growth. Academic Press, New York, 277-303.

McBean, D.M., Johnson, A.A. and Pitt, J.I. (1963). The absorption of sulfur dioxide by fruit tissue. J. Food Sci. 28, 257-265.

McBean, D.M., Pitt, J.I. and Johnson, A.A. (1965). Retention of absorbed sulfur dioxide in fruit tissue during drying. Food Techn. 19, 141-145.

McBean, D.M. (1967). Levels of free and combined sulphur dioxide in fruits during sulphuring and drying. Food Techn. 21, 1402-1406.

McWeeny, D.J., Biltcliffe, D.O., Powell, R.C.T. and Spark, A.A. (1969). The Maillard reaction and its inhibition by sulfite. J. Food Sci. 34, 641-643.

McWeeny, D.J., Knowles, M.E. and Hearne, J.F. (1974). The chemistry of non enzymic browning in foods and its control by sulphites. J.Sci.Food Agric. 25, 735-746.

McWeeny, D.J. (1979). The chemical behaviour of food additives. Proc. Nutr. Soc. 38, 129-133.

McWeeny, D.J. Shepard, M.J. and Bates, M.L. (1980). Physical loss and chemical reactions of SO_2 in strawberry jam production. J. Food Technol. 15, 613-617.

McWeeny, D.J. (1981). Sulfur dioxide and the Maillard reaction in food. Prog. Fd. Nutr. Sci. Vol. 5, 395-404.

Menzel, A.E.O. Wintersteiner, O. and Hoogerheide, J.C. (1944). The isolation of gliotoxin and fumigacin from culture filtrates of *Aspergillus fumigatus*. J. Biol. Chem 152, 419.

Miller, M.W. & Mrak, E.M. (1953). Yeasts associated with dried fruit beetles in figs. Appl.Microbiol. 1, 174-178.

Miller, M.W. & Chichester, C.O. (1960). The constituents of the crystalline deposits on dried fruit. Food Res. 25, 424-428.

Miller, M.W. & Phaff, H.J. (1962). Successive microbial population in Calimyrna figs. Appl.Microbiol. 10, 394-400.

Miller, M.W. & Tanaka, H. (1963). Microbial spoilage of dried prunes. III. Relation of equilibrium relative humidity to potential spoilage. Hilgardia 34, 183-190.

Moerck, K.E., McElfresh, P., Wohlman, A. and Hilton, B.W. (1979). Aflatoxin degradation in corn using sodium bisulphite, sodium hydroxide and aqueous ammonia. J. Food Prot. 43, 571.

Mohamed, M.S. & Mrak, E.M. (1942). Relation of variety and stage of development to composition of figs. Food Res. 7, 495-502.

Monier-Williams, G.W. (1927). The determination of sulphur dioxide in foods. Analyst 52, 415-416.

Moreau, C. & Moss, M. (1979). Moulds, toxins and food. John Wiley & Sons, Chichester, 43.

Morris, J.R., Cawthon, D.L. and Fleming J.W. (1979). Effects of temperature and SO_2 addition on quality and postharvest behavior of mechanically-harvested juice grapes in Arkansas. J. Am. Soc. Hortic. Sci. 104, 166-169.

Morton, S.G., Eadie, T. and Llwellyn, G.C. (1979). Aflatoxigenic potential of dried figs, apricots, pineapples and raisins. J.Assoc.Off.Anal.Chem. 62(4), 958-962.

Moss, M.O. (1989). Mycotoxins of *Aspergillus* and other filamentous fungi:review. J. Appl. Bact. Sympos. Suppl. No 18, 69S-81S.

Moss, M.O. (1995a). Mycotoxins in foods, feeds and pastures. Biodeterioration Abstracts 9(3), 259-264.

Moss, M.O. (1995b). The occurrence and significance of mould toxins (mycotoxins) in food. Food Science and Technology Today 9(1), 35-38.

Moss, M.O. (1996). Mycotoxins:Centenary Review. Mycol. Res. 100(5), 513-523.

Moss, M.O., Badii, F., Clifford, M.N. (1990). Reduced aflatoxin production by *Aspergillus parasiticus* after growth on a caffeine-containing medium. Letters in Applied Microbiology 10, 205-207.

Moss, M.O. & Frank, J.M. (1985a). Influence of the fungicide tridemorph on T-2 toxin production by *Fusarium sporotrichioides.* Transactions of the British Mycological Society 84, 585-590.

Moss, M.O. & Frank, J.M. (1985b). The influence on mycotoxins production of interactions between fungi and their environment in "Trichothenes and other mycotoxins" , ed. J.Lacey, John Wiley & Sons, Chichester, 257.

Moss, M.O. & Frank, M. (1987). Prevention:effects of biocides and other agents on mycotoxin production. In Natural Toxicants in Foods. Watson, D.H. (ed.) pp.231-251, Chichester, U.K.

Moss, M.O. & Frank, M. (1988). Variability in the production of trichothecenes by *Fusarium sporotrichioides*. International Biodeterioration 24(4/5), 445-453.

Moss, M.O. & Smith, J.E. (1985). Mycotoxins:formation, analysis and significance. John Wiley & Sons, Chichester, 7.

Moysidis, A. (1957). A new simple and rapid method for the determination of moisture in sultana raisins. Chimica Chronica. 22A, 143-147.

Mrak, E.M., Phaff, H.J., Vaughn, R.H. and Hansen, H.N. (1942a). Yeasts occurring in souring figs. J.Bacteriol. 44,441-450.

Mrak, E.M., Fischer, C.D. and Bornstein, B. (1942b). The effect of certain substances and pre-treatments on the retention of color and sulfur dioxide by cut fruits. Fruit Products J. 21, 297-299.

Nakazato, M., Morozumi, S., Saito, K., Fujinuma, K. Nishima, T. and Kasai, N. (1990). Interconversion of aflatoxin B_1 and aflatoxicol by several fungi. Appl. Environ. Microbiol. 56(5), 1465-1470.

Natarayan, C.P., Chari, C.N. and Mrak, E.M. (1948). Yeast population in figs during drying. Fruit Prod.J.Am.Food Manuf. 242-243.

Nelson, E.K. (1928). The acids of figs. J.Am.Chem.Soc. 50, 2012-2013.

Nelson, E.K. (1979). Harvesting and handling California table grapes for market. Agric.Sci.Publ. 4095, Univ. of California, Berkeley, 7-9, 48-49, 53-54.

Nelson, E.K. (1983). Effects of in-package sulfur dioxide generators, package liners, and temperature on decay and dessiccation of table grapes. Am. J. Enol. Vitic. 34, 10-16.

Nelson, P.E., Desjardins, A.E & Plattner, R.D. (1993). Fumonisins, mycotoxins produced by *Fusarium* species, biology, chemistry and significance. A. Rev. Phytopath. 31, 233-252.

Nichols, P.F. & Christie, A.W. (1930). Drying cut fruits. Bulletin 485, University of California.

Nichols, P.F. & Reed, H.M. (1931). What happens in the tropics. Western Canner and Packer 23, 11-15.

Nichols, P.F. & Reed, H.M. (1932). Experiments in harvesting and drying figs. Fruit Prod.J. 11, 237-240.

Nichols, P.F. (1933). Methods of sun drying fruits. Circ. 75, Univ. of Calif.

Nichols, P.F., Mrak, E.M. and Bethel, R. (1938). Effect of drying and storage conditions on color and sulfur dioxide retention of dried apricots. Food Research 4, 67-74.

Northolt, M.D., Van Egmond, H.P. and Paulsch, W.E. (1977). Differences between *Aspergillus flavus* strains in growth and aflatoxin B_1 production in relation to water activity and temperature. J.Food Prot. 40, 778.

Northolt, M.D., Verhulsdonk, C.A.H., Soentoro, P.S.S. and Paulsch, W.E. (1976). Effects of water activity and temperature on aflatoxin production by *Aspergillus parasiticus.* J.Milk Food Technol. 39, 170.

Nury, F.S., Taylor, D.H. and Brekke, J.E. (1960). Research for better quality in dried fruits. Figs. U.S. Dept.Agric. ARS-74-16.

Nury, F.S. & Bolin, H.R. (1965). Collaborative study on assay of sulfur dioxide in dried fruit. J. Assoc. Off. Agric. Chem. 48(4), 796-801.

Ough, C.S. (1983). Sulfur dioxide and sulfites. In "Antimicrobials in Foods" ed. A.L. Branen, P.M. Davidson. New York-Dekker, 177-203.

Ozay, G., Aran, N. & Pala, M. (1995). Influence of harvesting and drying techniques on microflora and mycotoxin contamination of figs. Nahrung 39(2), 156-165.

Pala, M. & Saygi, Y.B. (1990). Effect of different pretreatments on the quality of frozen figs. Prog. Sci. Techn. of Refrig. in Food Eng., Inst. Intern. du Froid, 595-601.

Pantastico, B. (1975). Structure of fruits and vegetables. In "Postharvest physiology, handling and utilisation of tropical and subtropical fruits and vegetables". AVI Publishing Westport Conn.

Papaioannou, A. (1989). Physicochemical properties of greek figs var. Kymi. Personnal communication.

Park, K.Y. & Bullerman, L.B. (1983). Effects of substrate and temperature on aflatoxin production by *Aspergillus parasiticus* and *Aspergillus flavus.* J. Food Prot., 46, 178.

Pattee, H.E. (1969). Production of aflatoxins by *Aspergillus flavus* cultured on flue-cured tobacco. Appl. Microbiol. 18, 952-953.

Pellicano, A. (1907). Il fico nel circondario de Gerace. Bol.Arbor.Ital.Anno. 3, 122-150.

Perfetti, G.A., Joe, F.L.Jr and Diachenko, G.W. (1989). Liquid chromatographic determination of sulfite in grapes and selected grape products. J. Assoc. Off. Anal. Chem. 72(6), 903-906.

Phaff, H.J., Mrak, E.M., Alleman, R. and Whelton, R. (1946). Microbiolgy of prunes during handling and drying. Fruit Prod. J. and Amer. Food Manufacture 25, 140-141, 155.

Phaff, H.J. & Miller, M.W. (1961). A specific microflora associated with the fig-wasp, *Blastophaga psenes Limnaeus.* J.Insect Pathol. 3, 233-243.

Phillips,R.J., Smith, E.H. and Smith, R.E. (1925). Fig smut. Calif. Agr.Expt.Stat.Bull. 387, 1-38.

Pitt, J.I. (1989a). Food mycology - an emerging discipline. J. Appl. Bact. Symp. Suppl. No 18, 000S.

Pitt, J.I. (1989b). Recent developments in the study of P*enicillium* and A*spergillus* systematics. J. Appl. Bact. Symp. Suppl. No 18, 37S-45S.

Pitt, J.I., Hocking, A.D. and Glenn, D.R (1983). An improved medium for the detection of *Aspergillus flavus* and *Aspergillus parasiticus*. J. Appl. Bact. 54, 109-114.

Ponting, J.D. (1944). Catechol test for frozen fruits. Quick Frozen Foods 7(5), 31, 46.

Ponting, J.D. & Johnson, G. (1945). Determination of sulfur dioxide in fruits. Ind. Eng. Chem., Anal. Ed. 17, 682-686.

Ponting, J.D., Jackson, R. and Watters, G. (1971). Refrigerated apple slices: Effects of pH, sulfites and calcium on texture. J. Food Sci. 36, 349-350.

Pontikis, C. (1987). Fig-tree. In "Specific Arboriculture", Caraberopoulos Publ.Co. Athens, 407-431.

Puech, A.A., Rebeiz, C.A., Catlin, P.B. and Crane, J.C. (1975). Characterisation of anthocyanins in fig *(ficus carica L.)* fruits. J. Food Sci. 40, 775-779.

Puech, A.A., Rebeiz, C.A. and Crane, J.C. (1976). Characterization of major plastic pigments in skin of Mission fig fruits. J. Am. Soc. Hort. Sci. 101, 392-394.

Pursuglove, J.W. (1968). *Ficus L.* In "Tropical crops" Vol.II, Longmans ,London, 386-390.

Rabie, C.J. & Smalley, E.B. (1965). Influence of temperature on the production of aflatoxin by *Aspergillus flavus*. Proc. Sympos. on Mycotoxins in Foodstuffs. Dept. Agric. Tech. Service Pretoria SA, Session 2, 18-29.

Rahn, O. & Cohn, J.E. (1944). Effect of increase in acidity on antiseptic efficiency. Ind. Eng. Chem. 36, 185-187.

Ramakrishna, Y., Bhat, R.V. and Vasanthi, S. (1990). Natural occurrence of mycotoxins in staple foods in India. J. Agric. Food Chem. 38(9), 1857-1859.

Raper, K.B. & Fennell, D.I. (1965). The genus *Aspergillus*. The Williams & Wilkins Co.

Reichert, N., Steimeyer, S. and Weber, R. (1988). Determination of aflatoxin B_1 in dried figs by visual screening, thin layer chromatography and ELISA. Zeit. Lebens. Unters. Forsch. 186(6), 505-508.

Reiss, J. (1975). Mycotoxins in foodstuffs. Eur. J. Appl. Microbiol. 2, 183.

Resnik, S., Neira, S., Pacin, A., Martinez, E., Apro, N. & Latreite, S. (1996). A survey of the natural occurrence of aflatoxins and zearalenone in Argentine field maize: 1983-1994. Food Addit. Contam. 13(1), 115-120.

Rheeder, J.P., Marasas, W.F.O., Thiel, P.G., Sydenham, E.W., Shephard, G.S., Schalkwyk, D.J. (1992). *Fusarium moniliforme* and fumonisins in corn in relation to human esophageal cancer in Transkei. Phytopathology 82(3), 353-357.

Rixford, G.P. (1918). Smyrna fig culture. U.S.D.A. Bull. 732, 1-43.

Rhem, H.J. (1964). Microbial inhibition in foods. ed. N.Molin. Almqvist and Wiksell, Gotenborg, 105-115.

Rhem, H.J. & Wittmann, H. (1962). Beitrug zur Kenntis der antimikrobiellen wirkung der schwefligen saure. Zeit.Lebensm. Unters.Forsch. 118, 413.

Roberts, A.C. & McWeeny, D.J. (1972). The uses of sulphur dioxide in the food industry. J. Food Techn. 7, 221-238.

Rockland, L.B. & Nishi, S.K. (1980). Influence of water activity on food product quality and stability. Food Techn. 34(4), 42-51.

Rodricks, J.V., Lustig, E., Campbell, A.D., and Stoloff, L. (1968). Aspertoxin, a hydroxy derivative of O-methyl sterigmatocystin from aflatoxin-producing cultures of *Aspergillus flavus.* Tedrahedron Lett. 25, 2975.

Roy, A.K. & Chourasia, H.K. (1990). Inhibition of aflatoxins production by microbial interaction. J. Gen. Appl. Microbiol. 36, 59-62.

Sahasrabudhe, M.R., Larmond, E. and Nunes, A.C. (1976). Sulfur dioxide in instant mashed potatoes. Can. Inst. Food Sci. Technol. 9, 207-211.

Salem, S.A. & Abdul-Nour, B.A. (1979). Sugars and amino acids in dried Iraqi figs. J. Sci. Food Agric. 30(6), 620-622.

Salunkhe, D.K. & Desai, B.B. (1984). Postharvest Biotechnology of fruits. Vol. II. "Chapter 6:Fig". pp.47-52. CRC Press Inc. Boca Raton, Florida.

Samarajeewa, U., Sen, A.C., Cohen, M.D. and Wei, C.I. (1990). Detoxification of aflatoxins in foods and feeds by physical and chemical methods. J. Food Prot. 53(6), 489-501.

Samson, R.A. & Pitt, J.I. (1985). Advances in *Penicillium* and *Aspergillus* Systematics. New York: Plenum Press.

Sanders, T.H., Davis, N.D. and Diener, U.L. (1968). Effect of carbon dioxide, temperature and relative humidity on production of aflatoxin in peanuts. J. Amer. Oil Chem. Soc. 45, 683-685.

Sanchis, V. Sala, N., Palomes, A. Santamarina, P. and Burdaspal, P.A. (1986). Occurrence of aflatoxin and aflatoxigenic molds in foods and feed in Spain. J. Food Prot. 49(6), 445-448.

Saravakos, G.D., Tsiourvas, D.A. and Tsami, E. (1986). Effect of temperature on the water adsorption isotherms of Sultana raisins. J. Food Sci. 51(2), 381-383, 387.

Sayavedra, L.A. & Montgomery, M.W. (1983). Effect of moisture, temperature and sulfur dioxide on color of dried apples. Ann. IFT Meet. 43rd Abstr. No 301.

Schelhorn, M.V. (1951). Untersuchungen uber Konservierungsmittel. VII. Wirksamkeit und wirkungsbereich der schwefligen saure. Dsch. Lebensm. Rundschau 47, 170.

Schindler, A.F., Palmer, J.G. and Eisenberg, W.V. (1967). Aflatoxin production by *Aspergillus flavus* as related to various temperatures. Appl. Microbiol. 15, 1006-1009.

Schroeder, H.W. & Hein, H. (1967). Aflatoxins:production of the toxins *in vitro* in relation to temperature. Appl. Microbiol. 15, 441.

Schroeter, L.C (1966). Sulfur dioxide. Applications in foods, beverages and pharmaceuticals. Pergamon, Lond island city, 191-227.

Schwimmer, S. (1980). Influence of water activity on enzyme reactivity and stability. Food Techn. 34(5), 64-74, 82.

Scott, P.M., Van Walbeek, W., Kennedy, B. and Anyeti, D. (1972). Mycotoxins (ochratoxin, citrinin and sterigmatocystin) and toxigenic fungi in grains and other agricultural products. J. Agric. Food Chem. 20, 1103.

Scott, P.M. (1989). The natural occurrence of trichothecenes. In Trichothecene Toxicosis : Pathophysiological Effects, Vol.1, Beasley, V.R. (ed.) pp.2-26, CRC Press, Boca Raton, Fl.

Sharma, A., Behere, S.R., Padwal-Desai and Nadkarni, G.B. (1980). Influence of inoculum size of *Aspergillus parasiticus* spores on aflatoxin production. Appl. Environ. Microbiol. 40, 989.

Sharman, M., Patey, A.L., Bloomfield, D.A. and Gilbert, J. (1991). Surveillance and control of aflatoxin contamination of dried figs and fig paste imported into the U.K. Food Add. Contam. 8(3), 299-304.

Sharman, M., Macdonald, S., Sharkey, A.J., & Gilbert, J. (1994). Sampling bulk consignments of dried figs for aflatoxin analysis. Food Addit. Contam. 11(1), 17-23.

Sherertz, P.C., Eadie, T., Young, J.W. and Llewellyn, G.C. (1976). Aflatoxin occurrence on raw and cooked soyabeans inoculated with three *Aspergillus* isolates. J. Assoc. Off. Anal. Chem. 59(3), 662-665.

Shih, C.N. & Marth, E.H. (1973). Release of aflatoxin from the mycelium of *Aspergillus parasiticus* into liquid media. Zeit. Lebensm. Untersch. Forsch. 152, 336.

Shipton, J. (1954). Estimation of sulphur dioxide in dried foods. Food Preserv. Quart. 14, 54-56.

Shorey, H.H., Ferguson, L. and Wood, D.L. (1989). Solar heating reduces insect infestations in ripening and drying figs. Hortscience 24(3), 443-445.

Shotwell, O.L., Hesseltine, C.W., Burmeister, H.R., Kwolek, W.F., Shannon, G.M. and Hall, H.H. (1969). A survey of cereal grains and soybeans for the presence of aflatoxin. II. Corn and soybeans. Cereal Chem. 46, 454-463.

Smith, R.E. & Hansen, H.N. (1927). The improvement of quality in figs. Univ.Calif.Agr.Expt.Stat.Circ. 311, 1-23.

Smith, R.E. & Hansen, H.N. (1931). Fruit spoilage diseases of figs. California Agr.Expt.stat.Bull. 506, 1-84.

Snijders, C.H.A. (1994). Breeding for *Fusarium* resistance in wheat and maize. In *Mycotoxins in Grain.* Miller, J.D. & Trenholm, H.L (eds.) pp.37-58, Eagan Press, st Paul, MN.

Society of Dyers and Colourists (1971). Colours Index Vol.2, 3rd edn. The Society of Dyers and Colourists, Bradford.

Sommer, N.F., Buchanan, J.R. and Fortlage, R.J. (1974). Production of patulin by *Penicillium expansum.* Appl. Microbiol. 28, 589.

Souci, S.W. (1951). Zur Frage der chemischen Konservierung der Lebensmittel. Z. Lebensm.-Untersuch. u. Forsch. 93, 37-51, 61-72.

Sreenivasamurthy, V., Parpia, H.A.B., Srikanta, S. and Shankar, M. (1967). Detoxification of aflatoxin in peanut meal by hydrogen peroxide. J. Assoc. Off. Anal. Chem. 50(2), 350-354.

Stadtman, E.R, Barker, H.A., Haas, V. and Mrak E.M. (1946a). Storage of dried fruit: influence of temperature on deterioration of apricots. Ind.Eng.Chem. 38(50), 541-543.

Stadtman, E.R, Barker, H.A., Haas, V., Mrak E.M. and Mackinney, G. (1946b). Storage of dried fruit:gas changes during storage of dried apricots and influence of oxygen on rate of deterioration. Ind.Eng.Chem. 38(3), 324-329.

Stadtman, E.R, Barker, H.A., Mrak E.M. and Mackinney, G. (1946c). Storage of dried fruit. Influence of moisture and sulphur dioxide on deterioration of apricots. Ind.Eng.Chem. 38(3), 99-104.

Stadtman, E.R. (1948). Non-enzymatic browning in fruit products. Advances in Food Research 1, 325-372.

Stafford, A.E., Bolin, H.R. and Mackey, B.E. (1972). Absorption of aqueous bisulfite by apricots. J. Food Sci. 37, 941-943.

Steering Group on Food Surveillance (1988). The twenty fourth report of the Steering Group on Food Surveillance, Paper no 18, Publ. HMSO, London.

Steiner, W., Brunschweiler, K., Leimbacher, E., Schneider, R. (1993). Aflatoxin B_1 and G_1, cyclopiazonic acid, Kojic acid and ochratoxin A in dried figs showing BGY-fluorescence. Mitt. Geb. Lebens. Hyg. 84(5), 523-536.

Steiner, W.E., Rieker, R.H. and Battaglia, R. (1988). Aflatoxin contamination in dried figs: distribution and association with fluorescence. J. Agr. Food Chem. 36(1), 88-91.

Storey, W.B. (1976). Figs. In "Evolution of crop plants" ed. N.W. Simmons, Longman, London, 205-208.

Subcommittee on Review of the GRAS List (1972). A comprehensive survey of industry on the use of food chemicals generally recognised as safe (GRAS). Committee on Food Protection, Division of Biology and Agriculture, National Research Council, National Academy of Sciences, Washington D.C.

Sullivan, J.J., Hollingworth, T.A., Wekell, M.M., Newton, R.T. and Larose, J.E. (1986). Determination of sulfite in food by flow injection analysis. J. Assoc. Off. Anal. Chem. 69, 542-546.

Suzuki, H. (1977). Comparison of several methods for determination of sulphites. J. Food Hyg. Soc. Jpn. 18(3), 290-295.

Sydenham, E.W., Westhuizen, L., van der Stockenstrom, S., Shephard, G.S., Thiel, P.C. (1994). Fumonisin-contaminated maize: physical treatment for the partial decontamination of bulk shipments. Food Additives and Contaminants 11, 25-32.

Tabak, H.H. & Cooke, W.B. (1968). The effect of gaseous environment on the growth and metabolism of fungi. Bot. Rev. 34, 124.

Tabata, S., Kamimura, H., Ibe, A., Hashimoto, H., Tamura, Y. and Nishima, T. (1992). Fate of aflatoxins during cooking process and effect of food components on their stability. J. Food Hyg. Soc. Japan. 33(2), 150-156.

Tabata, S., Kamimura, H., Ibe, A., Hashimoto, H., Iida, M., Tamura, Y. & Nishima, T. (1993). Aflatoxin contamination in Foods and Foodstuffs in Tokyo: 1986-1990. J. Assoc. Off. Anal. Chem. International 76(1), 32-35.

Tanner, F.W. (1944). The Microbiology of Foods. Garrard Press, Champaign, Illinois, 633.

Tapia de Daza, M.S. & Beuchat, L.R. (1992). Suitability of modified dichloran-glycerol (DG18) agar for enumerating unstressed and stressed xerophilic molds. Food Microbiol. 9(4), 319-333.

Tarter, E.J., Hanchan, J. and Scott, P.M. (1984). Improved liquid chromatographic method for determination of aflatoxins in peanut butter and other commodities. J. Assoc. Off. Anal. Chem. 67, 597-600.

Taylor, S.L., Higley, N.A. and Bush, R.K. (1986). Sulfites in foods: uses, analytical methods, residues, fate, expossure assessment, metabolism, toxicity and hypersensitivity. Advances in Food Research 30, 1-76.

Thewlis, B.H. & Wade, P. (1974). An investigation into the fate of sulphites added to hard sweet biscuit doughs. J. Sci. Food Agric. 25, 99-105.

Thiselton - Dyer (1876). British popular customs present and past. G.Bell and sons, London.

Troller, J.A. & Christian, J.H.B. (1978). Water activity and food. New York: Academic Press.

Troller, J.A. (1980). Influence of water activity on microorganisms in foods. Food Technol. 34(5), 76-80, 82.

USDA (U.S. Dept. Agr.) (1978). Agricultural statistics. USDA GOV. no.7, Printing Office, Washington D.C.

Vanderzant, C. & Splittstoesser, D.F. (1993). Compendium of methods for the microbiological examination of foods. 3rd ed. American Public Health Association.

Van Egmond, H.P. (1989). Current situation on regulations for mycotoxins. Overview of tolerances and status of standard methods of sampling and analysis. Food Additives and Contaminants 6, 139-188.

Van Walbeek, W., Clademenos, T. and Thatcher, F.S. (1969). Influence of refrigeration on aflatoxin production by strains of *Aspergillus flavus*. Can. J. Microbiol. 15, 629.

Vas, K. (1949). The equilibrium between glucose and sulfurous acid. J. Soc. Chem. Ind. 68, 340-343.

Vas, K. & Ingram, M. (1949). Preservation of fruit juices with less sulfur dioxide. Food Manufacture 24, 414-416.

Vining, L.C. (1992). Role of secondary metabolites from microbes. In Secondary Metabolites: Their Function and Evolution (ed. D.J. Chadwick & J. Whelan) pp. 184-194. John Wiley: Chichester.

Viquez, O.M, Castell - Perez, M.E, Shelby, R.A. & Brown, G. (1994). Aflatoxin contamination in corn samples due to environmental conditions, aflatoxin - producing strains and nutrients in grain grown in Costa - Rica. J. Agric. Food. Chem. 42(11) 2511-2555.

von Schelhorn, M. (1953). Efficacy and specificity of chemical food presevatives. Food Technol. 7, 47-101.

Walker, R. (1984). Biological and toxicological consequences of reactions between sulphur (IV) oxo-anions and food components. Food Chem. 15, 127-138.

Walti, A. (1938). Crystalline Ficin. J. Am. Chem. Soc. 60, 493.

Walther, H.J., Parker, C.E., Harvan, D.J., Voyksner, R.D., Hernandez, O., Hagler, W.M., Hamilton, P.B. and Hass, J.R. (1983). Fast atom bombardment mass spectrometry of aflatoxins and reaction products of sodium bisulfite with aflatoxins. J. Agric. Food Chem. 31, 168-170.

Warmbier, H.C., Schnickles, R.A. and Labuza, T.P. (1976). Effect of glycerol on nonenzymatic browning in a solid intermediate moisture model food system. J. Food Sci. 41, 528.

Warner, C.R., Daniels, D.H., Pratt, D.E., Joe, F.L.Jr, Fazio, T. and Diachenko, G.W. (1987). Sulphite stabilization and high performance liquid chromatographic determination: a reference method for free and reversibly bound sulphite in food. Food Additives and Contaminants 4, 437-445.

Wedzicha, B.L. (1984). Sulphur dioxide in foods-chemical interactions. BNF Nutrition Bulletin, 9(3), 155-164.

Wedzicha, B.L, Lamikanra, O., Herrera, J.C. and Panahi, S. (1984). Recent developments in the understanding of the chemistry of sulphur (IV) oxospecies in dehydrated vegetables. Food Chem. 15, 141-155.

Wei, D.L. & Jong, S.C. (1986). Production of aflatoxins by strains of the A.flavus group maintained in ATCC. Mycopathologia 93, 19-24.

Weisser, H., Weber, J. and Loncin, M. (1982). Water vapor sorption isotherms of sugar substitutes in the temperature range 25° to 80°C. Intern. Zeits. Lebens. Technol. Verfahrenstechnik 33(2), 89.

Wehner, F.C. & Rabie, C.J. (1970). Efficacy and specificity of chemical food preservatives. Phytophylactica 2, 165-170.

Wenk, P., Winger, W., Zurcher, K., Stutz, W. (1994). A new proposal for the control of aflatoxins in dried figs. Mitt. Geb. Lebens. Hyg. 85(6), 737-750.

White, E.C. (1940). Bactericidal filtrates from a mold culture. Science 92, 127.

Wicklow, D.T., Shotwell, O.L. and Adams, G.L. (1981). Use of aflatoxin-producing ability medium to distinguish aflatoxin-producing strains of *Aspergillus flavus*. Appl. Environ. Microbiol. 41, 697-699.

Wildman, J.D., Stoloff, L. and Jacobs, R. (1967). Aflatoxin production by a potent *Aspergillus flavus Link* isolate. Biotechnol.Bioeng. 9, 429-437.

Wiley, H.W. (1907). Influence of food preservatives and artificial colors on digestion and health. III. Sulphurous acid and sulphites. U.S. Dept. Agr. Bur. Chem. Bull., 84 (Part 3).

Williams, K.T., Potter, E.F. and Bevenue, A. (1952). A study, by paper chromatography, of the occurrence of non-fermentable sugars in plant materials. J. Assoc. Off. Agric. Chem. 35, 483-486.

Wilson, D.M. & Jay, E. (1975). Influence of modified atmosphere storage on aflatoxin production in high moisture corn. Appl. Microbiol. 29, 224-228.

Wilson,D.M., Jay, E. Hale, O.M. and Huang, L.H. (1977). Controlled atmosphere storage of high-moisture corn and peanuts to control aflatoxin production. Ann. Technol. Agr. 27, 339-342.

Wilson, T.J. & Romer, T.R. (1991). Use of the mycosep multifunctional cleanup column for liquid chromatographic determination of aflatoxins in agricultural products. J.Assoc. Off. Anal. Chem. 74(6), 951-956.

Winton, A.L & Winton, K.B. (1935). The structure and composition of foods Vol.II., ed. A.L. Winton. J.Wiley and sons Inc., New York.

Woodroof, J.G. & Cecil, S.R. (1945). Sulfur dioxide solution as preservative for fruits and vegetables. Georgia Agric.Expt.Stat.Bull. 238, 1-32.

Woodroof, J.G. & Luh, B.S. (1975). Commercial fruit processing. The AVI Publishing Co. Inc. Westport Connecticut.

Working Group on Toxins (1982). Determination of the Aflatoxins B_1, B_2, G_1 and G_2. Mitt. Geb. Lebensmittelunters Hyg. 73, 362-367.

Yabuta, T. (1924). The constitution of kojic acid, a gamma-pyrone derivative formed by *Aspergillus oryzae* from carbohydrates. J. Chem. Soc. 125, 575.

Yagen, B., Hutchins, J.E., Cox, R.H., Hagler, W.M. Jr and Hamilton, P.B. (1989). Aflatoxin B_1S: Revised structure for the sodium sulfonate formed by destruction of aflatoxin B_1 with sodium bisulfite. J. Food Prot. 52(8), 574-577.

Yoshizawa, T., Yamashita, A., Luo, Y. (1994). Fumonisin occurrence in corn from high- and low-risk areas for human oesophageal cancer in China. Appl. Envir. Microbiol. 60, 1626-1629.

Yousef, A.E. & Marth, E.H. (1981). Growth and synthesis of aflatoxin by *Aspergillus parasiticus* in the presence of sorbic acid. J. Food Prot. 44, 736.